LIFE-CYCLE STUDIES ON STRIGEOID TREMATODES

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SUMMARY

Between 1971 and 1973, studies were carried out on the taxonomy, life-cycles, and ecology of a number of strigeoid trematodes occurring in Scotland and in Iceland.

Adults of the following species were obtained from Scotland by feeding their metacercariae to ducklings or to black-headed gulls; Apatemon (A.) gracilis (Rudolphi, 1819) Szidat, 1928, Apatemon (Australapatemon) minor Yamaguti, 1933, Cotylurus (C.) cornutus (Rudolphi, 1808) Szidat, 1928, Cotylurus (Ichthyocotylurus) variegetus (Creplin, 1825) Szidat, 1928 sensu Odening and Bockhardt, 1971, Diplostomum (D.) spathaceum (Rudolphi, 1819) Braun, 1893, Diplostomum (D.) phoxini (Faust, 1918) Arvy and Buttner, 1954, Diplostomum (D.) gasterostei Williams, 1966 and Diplostomum (D.) sp.

Metacercariae from freshwater snails and fish in Iceland were fed to ducklings, and yielded the strigeoids Apatemon gracilis, Cotylurus cornutus, and an unidentified Diplostomum sp. Trematodes of other families were also obtained.

Amongst strigeoid cercariae emerging from Lymnaea peregra in Scottish waters were the cercariae of Apatemon gracilis and of four species of Diplostomum (Diplostomum spathaceum, D. phoxini, D. gasterostei, and Diplostomum sp.). The morphology of these cercariae was described, and features of taxonomic value discussed with special reference to cercaria of the subgenus Diplostomum. The Diplostomum cercariae studied could be separated on the basis of their resting posture and number of caudal bodies, with details of spination a secondary character.

The development and structure of the metacercaria of Apatemon gracilis was described. The process of excystation of the fully developed metacercaria was compared with that in other species. Experimental fish host and location specificities of the cercaria and metacercaria of Apatemon gracilis and of the above Diplostomum spp. were compared with the natural occurrence of the metacercariae.

Experiments were carried out on factors affecting the development and hatching of eggs of a representative strigeoid (Diplostomum spathaceum). These revealed that eggs are capable of hatching under all conditions of pH and salinity in which snail hosts are likely to occur. Their development rate appears negligible below 10°C, but increases exponentially with temperature to a maximum at about 30°C. The eggs are capable of hatching in the dark, of surviving long periods at low temperatures, and of developing at low oxygen concentrations

The life-cycle of Lymnaea peregra in a Scottish trout farm was followed during 1972 and 1973. During 1972 there were peaks of egg laying by the snails in May and in August. Young snails appeared in the population after each peak. During 1973 a single period of egg laying occurred, with a peak in June. Climatic factors may have been responsible for the differing life-cycles in these two years. Each peak of egg laying was followed by the death of the older snails. at the end of a breeding period, few large, old snails could be found, and these were generally infected with rediae or sporocysts. Infected snails appeared to live longer and grow larger than uninfected. The first increase in infection levels

of strigeoid metacercariae in fish occurred in June in 1972 and 1973. Cercariae emerging from old snails infected the previous year were responsible for this. It appeared that, when the snails reproduced sufficiently early in the year, trematode infections in the young snails could mature before the water temperature fell in the autumn. Cercariae from these snails may have been responsible for the second increase in fish infection levels late in the summer.

KEY TO ABBREVIATIONS

AO	anterior organ	Ov	ovary
AT	apical tuft	P	pharynx
BA	bladder arm	PC	parasite cyst
Bl	bladder	PG	penetration gland
Ca	gut caecum	PGD	penetration gland duct
CB	caudal body	Pm	pericardium
CC	cluster of cilia	PrG	proteolytic gland
E	embryo	POC	post-oral collar
FB	forebody	RG	rudiments of genitalia
Fc	flame cell	SV	seminal vesicle
GC	genital cone	T_1, T_2	testis (1st or 2nd)
GP	genital pore	UES	unpigmented eyespot
HB	hindbody	Ut	uterus
HF	holdfast	V	vitellaria
L	lappet	VC	vitelline cells
M	mouth opening	Ve	ventricle
O	oesophagus	VS	ventral sucker
Op	operculum	Z	zygote
OS	oral sucker		

GENERAL INTRODUCTION

During recent summers many cases of blindness in brown and rainbow trout were observed at College Mill Trout Farm in Perthshire. Examination of blind fish revealed a heavy infection of the eyes with trematode metacercariae or "eyeflukes". The term "eyefluke" as used here, refers to metacercariae of certain members of the superfamily Strigeoidea Railliet, 1919 (sensu Dubois, 1964; 1970b). The adjective "strigeoid" should be used for the group in preference to the more common "strigeid", the latter suggesting that members of only one family, the Strigeidae, are under discussion.(Hoffman, 1960).

Strigeoids may be defined as follows: Digenetic trematodes having a life-cycle involving three or four hosts. Adult worms occur in the digestive tract of reptiles, birds and mammals. The adult is divided into two distinct segments, the forebody bearing oral and ventral suckers and the characteristic holdfast organ (concerned with attachment and nutrition) and the hindbody containing male and female reproductive systems with a terminal genital pore. Eggs passed in the faeces of their hosts embryonate in freshwater. Miracidia with two pairs of flame cells give rise to sporocyst generations in aquatic snails. Fork-tailed cercariae released from snails have long tail furcae, possess an anterior organ (bearing penetration gland duct and gut openings), a pharynx and a ventral sucker. From their non-epithelial excretory bladder two lateral collecting ducts run to the level of the ventral sucker where they divide into anterior and posterior ducts draining flame cells in the body and tail-stem. The bladder discharges into a caudal excretory

canal, a distributary of which opens to the exterior midway along each furca. Metacercariae (or mesocercariae requiring ingestion by a further host for development to metacercariae) develop from actively penetrating cercariae in a variety of invertebrate and vertebrate hosts. In structure the fully developed metacercaria resembles an adult forebody with genital primordia in a rudimentary hindbody.

A) History and classification of adult strigeoids.

Records of adult strigeoids date back to the early days of Linnean systematics. Planaria alata vel dubia Goeze, 1782 was redescribed by Schrank, 1788 as the type of his new genus, Alaria. One other genus, Strigea Abildgaard, 1790 was proposed in the eighteenth century. Many new digenetic trematodes were described in the early years of the nineteenth century. The characteristic appearance of the strigeoids led Rudolphi in 1819 to place the majority of known forms in his genus Amphistoma. Dujardin (1845) grouped most strigeoids in the genus Holostomum Nitzsch, 1816. Diesing (1850) considered that adult strigeoids fell into the genera Holostomum Nitzsch, 1816, Hemistomum Diesing, 1850 and Eustemma Diesing, 1850. Brandes (1890) created the family Holostomidae to include the subfamilies Diplostomeae Hemistomeae and Holostomeae.

During the late nineteenth and early twentieth centuries there was much confusion concerning the position of the strigeoids within the trematodes. Von Linstow (1877) studied Holostomum cornucopia Molin, 1859 (=Strigea strigis (Schrank, 1788) Abildgaard, 1790). He considered that the miracidium of this species would

develop without sporocyst or cercarial generations into a metacercaria. Thus justified, several authors removed the strigeoids to the "Metastatica" considered to have life-cycles intermediate between those of digenetic and monogenetic trematodes. Faust (1917 p66) questioned this supposed "metastatic" development, but thought that metacercariae (of Cotylurus flabelliformis (Faust,1917) van Haitsma, 1931) developed from embryos within rediae without a free swimming cercarial stage.

The observations of Lutz (1921) finally established the strigeoids firmly within the digenetic trematodes. Working in Brazil, he demonstrated that three forms of fork-tailed cercariae would develop into metacercariae in their respective intermediate hosts, snails, leeches and tadpoles. These metacercariae gave rise to adults of Strigea spp. when fed to the appropriate final hosts. A number of authors during the 1920's confirmed that strigeoids are truly digenetic, having a cercaria corresponding to the longifurcate pharyngeate cercaria (see Dawes, 1946 p419 et seq).

Subsequently strigeoids were included in important classifications of the digenetic trematodes proposed by Poche (1926) and by La Rue (1926b, 1957). Sudarikov (1959, 1960a & b) gives an excellent historical review of the group.

At present slightly different classifications of the strigeoids are employed by Dubois (1953, 1964, 1970b) and by Sudarikov (all references). These are compared with that of La Rue (1957) in Appendix 1. Within the superfamily Strigeoidea Railliet, 1919 Dubois (1970b) recognises the families Strigeidae Railliet, 1918; Diplostomatidae Poirier, 1886; Proterodiplostomatidae

Dubois, 1936 and Bolbocephalodidae Strand, 1935. Experimental completion of the life-cycle has only been achieved for members of the first two families (see Figures 1 & 2) and it is with these that the present study is concerned. Appendix 2 includes definitions of the Strigeidae and the Diplostomatidae and lists of their genera. In this study the classification of the Strigeidae and Diplostomatidae according to Dubois (1968a, 1970a) will be followed.

B) History and classification of metacercariae.

Strigeoid metacercariae can be readily classified in a small number of groups on morphological grounds. As many have been described without reference to their adult forms and as these distinct larval groups each contain members of several adult genera, it has remained convenient, if taxonomically questionable, to refer metacercariae to defined "larval genera".

Von Nordmann (1832) described several trematodes from the eyes of fish. He believed these to be adult worms, placing some in the existing genera Holostomum Nitzsch and Distoma Retzius, and erecting a new genus, Diplostomum for the remainder. Although he claimed to have distinguished 58 species within this latter genus, he only described two, Diplostomum volvens and D. clavatum representing slightly differing morphological groups. Steenstrup (1842) considered that much of the life-cycle of these eyeflukes occurs within the eye itself, Diplostomum volvens representing an adult form of which D. clavatum and Holostomum cuticola (Nordmann, 1832) were "larva" and "pupa" respectively. However his views were not generally accepted. Dujardin (1845) and others

considered that all these forms in the eye represented larval flukes.

The adult form of Diplostomum volvens was established in 1894 when Braun reported a series of feeding experiments by the Ehrhardt brothers. In one of these infected eyes of roach (Rutilus rutilus L.) were fed to a black-headed gull (Larus ridibundus L.) and adult specimens of Hemistomum spathaceum (Rudolphi, 1819) Diesing, 1850 were obtained. The genus Hemistomum Diesing, 1850 being a junior synonym of Alaria Schrank, 1788, La Rue (1926a) removed Hemistomum spathaceum to new genus which he proposed to call Proalaria. In the opinion of Hughes (1929a), the name Proalaria should be suppressed as a synonym of Diplostomum. Thus Hemistomum spathaceum (Rudolphi) Diesing, 1850 became Diplostomum spathaceum (Rudolphi) Braun, 1893.

As it was not certain that other members of von Nordmann's genus Diplostomum would prove congeneric with D. spathaceum, another generic name had to be found for the remaining larval species. That chosen was Diplostomulum, first suggested by Brandes (1892) and later adopted by other authors.(see Hughes, 1929a). Appendix 3 contains a definition of the larval genus Diplostomulum.

Metacercariae originally placed in the larval genera Tylodelphys Diesing, 1850 (type species T. clavata (von Nordmann)) and Codonocephalus Diesing, 1850 (type species C. urnigera (Rudolphi)) are now placed in Diplostomulum, the names Tylodelphys and Codonocephalus being reserved for adult genera (see Hughes, 1929a and Dubois, 1970a pp 261-262).

Steenstrup (1842) described Distoma tarda from

aquatic snails. De Filippi (1855) on finding similar forms in snails proposed the name Tetracotyle for this group. Diesing (1858) referred to these forms as Tetracotyle typica. Dujardin (1845 p 473) described without naming, similar forms from Lymnaea palustris Muller. He speculated that these might give rise to adult "holostomes" in the intestine of water birds eating the snail. This was later proved correct when Ercolani (1881) fed Tetracotyle to ducks. Faust (1918) compiled the first synopsis of described Tetracotyle species and defined the genus.

More recently the larval genus Neascus has been proposed by Hughes, 1927 of which N. cuticola (von Nordmann) is the earliest described form. See Appendix 3 for definitions of the larval genera.

C) Origins and aims of the present study.

Heavy infections of eyeflukes causing mass blindness of hatchery fish have been recorded many times (Ferguson and Hayford, 1941; Bauer, 1959 p 37; Shigin, 1965b). As a consequence of their blindness, fish are unable to feed normally and may lose condition and die. Concern over the economic effects of the eyefluke problem led the owner of College Mill Trout Farm to consult the Department of Fisheries and Agriculture for Scotland in 1969. Mr. A.D. Campbell of that Department has since followed the incidence of eyefluke there and brought it to the attention of this laboratory.

When the present study was initiated late in 1971, it was assumed that a single species, Diplostomum spathaceum, was responsible for the infection. However examination of trout from the trout farm suggested that metacercariae

of more than one species were present in the eyes. Furthermore, several morphologically distinct strigeoid cercariae emerged from Lymnaea peregra collected at the trout farm. Identification of the strigeoids present therefore became a prime objective. It was hoped that experimental completion of their life-cycles would yield sufficient data for this purpose.(Chapters 1&2).

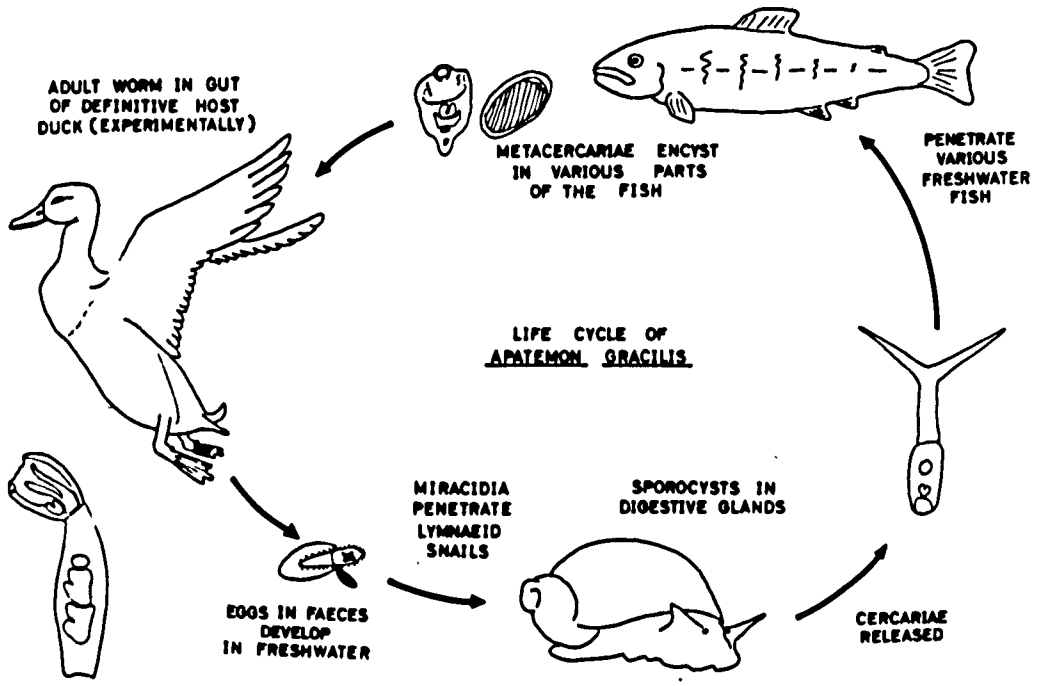
The snail populations at College Mill Trout Farm were sampled over a period of almost two years in an attempt to account for the seasonal pattern of fish infections noted by Campbell (pers. comm.)(see Chapter 4). This work, and studies on the developmental requirements of strigeoid eggs (Chapter 3), was intended to allow correlation of snail and trematode life-cycles and yield information on the epidemiology of the strigeoids.

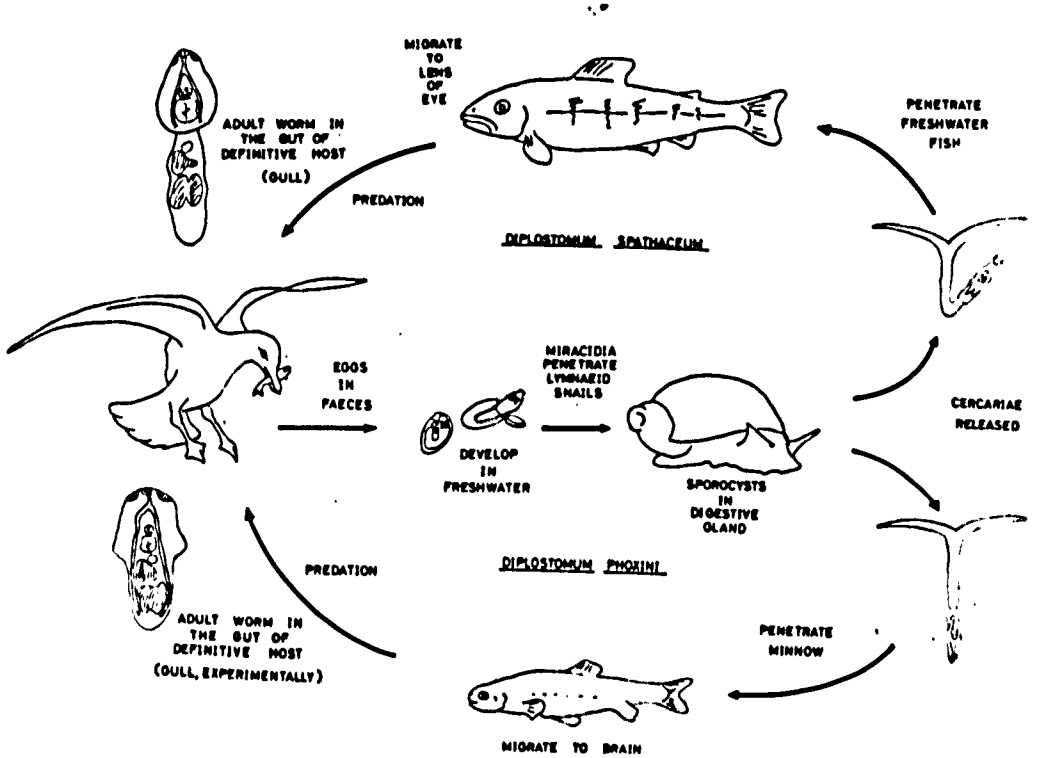
During the summer of 1972, the opportunity arose to study the strigeoid parasites in Iceland. Chapter 5 of this thesis is a paper discussing these.

facing page 8

Figure 1 The life-cycle of Apatemon (A.) gracilis (Rudolphi) Szidat, a member of the family Strigeidae.

Figure 2 The life-cycles of Diplostomum (D.) spathaceum (Rudolphi) Braun, and D. (D.) phoxini (Faust) Arvy and Buttner, members of the family Diplostomatidae.


ADULT WORM IN GUT OF DEFINITIVE HOST DUCK (EXPERIMENTALLY)
METACERCARIAE ENCYST IN VARIOUS PARTS OF THE FISH
PENETRATE VARIOUS FRESHWATER FISH
LIFE CYCLE OF APATEMON GRACILIS
MIRACIDIA PENETRATE LYMNAEID SNAILS
SPOROCYSTS IN DIGESTIVE GLANDS
EGGS IN FAECES DEVELOP IN FRESHWATER
CERCARIAE RELEASED


MIGRATE TO LENS OF EYE
ADULT WORM IN THE GUT OF DEFINITIVE HOST (GULL)
PREDATION
PENETRATE FRESHWATER FISH
DIPLOSTOMUM SPATHACEUM
EGGS IN FAECES
MIRACIDIA PENETRATE LYMNAEID SNAILS
DEVELOP IN FRESHWATER
SPOROCYSTS IN DIGESTIVE GLAND
CERCARIAE RELEASED
DIPLOSTOMUM PHOXINI
PREDATION
ADULT WORM IN THE GUT OF DEFINITIVE HOST (GULL, EXPERIMENTALLY)
PENETRATE MINNOW
MIGRATE TO BRAIN

CHAPTER 1 THE ADULT STRIGEOIDS

INTRODUCTION

The purpose of this chapter is to record the strigeoid species obtained as adults during this study and to discuss their history and some problems involved in their identification. A summary of the chapter may be found on page 24.

It has been the practice to differentiate strigeoid species by morphological features of adult worms rather than by details of life-cycles and larval stages. This approach has been forced upon systematists since the life-cycles of the majority of species in many genera are not yet known. Thus Dubois (1968a, 1970a) and Sudarikov (all references) in their monographs gave priority to adult morphology in species separation.

Identification of adult strigeoids on this basis is often very difficult (Vojtek and Vojtkova, 1971; Odening in discussion in Niewiadomska, 1964b; Niewiadomska, 1971a). Determination is often further hindered by the lack of uniform methods for preparing permanent material despite appeals to this effect by numerous authors. Ulmer (195) discussed the effects of flattening during fixation on dimensions and apparent topography of various organs of the brachylaemid trematode Postharmostomum helicis (Leidy, 1847) Robinson, 1949. Sinclair (1971) considered that failure to appreciate this source of artifact had led authors to propose three species which he then synonymised with the microphallid trematode Odhneria odhneri Travassos, 1921. Later, Sinclair and John (1973) advocated relaxation of live worms in hot water to give

uniform body shape prior to fixation.

Berrie (1960a) discussed the influence of the definitive host on the size of worms. Metacercariae of Diplostomum phoxini (Faust, 1918) which had matured in mice, ducklings, or herring gull chicks varied greatly in size according to their host. Campbell (1973) has demonstrated a similar phenomenon when metacercariae of Cotylurus flabelliformis (Faust, 1917) van Haitsma, 1931 were fed to different bird hosts and Palmieri (1973) has commented upon this with reference to Posthodiplostomum minimum (MacCallum, 1921) Dubois, 1936.

Dubois (discussion in Dubois, 1970b) has developed a system of classification based on both adult morphology and adult host specificity. He believes (Dubois, 1957) that the holdfast confers a more intimate association between strigeoids and their hosts than is usual among trematodes. By the same token, this physical and physiological intimacy would restrict any given strigeoid species to maturation only within a narrow range of hosts.

However, the taxonomic value of adult host specificity has been strongly questioned. Niewiadomska (1973) in analysing data on the natural occurrence of strigeoids in birds and mammals observed that "the range of specificity enlarges according to the number of records". Recent experimental work suggests that strigeoid host specificity in nature may owe more to the feeding habits of the final host than to the lack of adaptability of the parasite. Certainly, in vitro cultivation of metacercariae of Codonocephalus urniger (Rudolphi, 1819) Luhe, 1909 by Dollfus et al. (1956) and of Cotylurus lutzi Basch, 1969 by Basch et al. (1973) suggests that highly

specific conditions may not be neccessary for maturation and egg production in at least some strigeoids. Campbell (1973) has demonstrated that Cotylurus flabelliformis may mature experimentally in several orders of birds from which this parasite has not been recorded in nature. Palmieri (1973) expanding on earlier work by Ulmer (1961) and Campbell (1972), has experimentally extended the definitive host range of the "avian" strigeoid Posthodiplostomum minimum to include amphibians, reptiles and numerous orders of both birds and mammals.

MATERIALS AND METHODS

A) Sources and maintenance of birds.

In attempts to obtain adult worms strigeoid metacercariae were fed to birds of two species, the black-headed gull (Larus ridibundus L.) and the domestic duck (Anas platyrhyonchos dom.).

Black-headed gull chicks, not more than one week old, were obtained from a gull breeding colony in Perthshire by permission of the Nature Conservancy. The chicks were kept in a polythene tank, the bottom liberally covered with sawdust, and maintained at 20-22°C.. Perch, pike and roach obtained by gill netting from the Forth and Clyde Canal close to Glasgow were gutted and deep-frozen at -20°C. for some days to kill parasites. Gull chicks were offered this fish finely chopped and mixed with equal quantities of chicken broiler starter crumb (B.O.C.M. Ltd.). They rapidly learned to eat this mixture after some prompting (by presenting them with food on the end of a finger). After 2-3 weeks the chicks were capable of

feeding on chunks of fish alone. Water was supplied ad lib.. Before they were able to fly (at about 6 weeks) chicks were transferred to a large cage 1.5 by 1.5 by 1 m high, containing a tank with a constant supply of cold water.

Ducklings were obtained from a commercial breeder at between 1 and 7 days of age. They were maintained at 20-22°C. and fed chicken broiler starter crumb mixed to a paste with water. Clean water was supplied ad lib. in shallow dishes.

B) Experimental infection of birds.

Prior to feeding with metacercariae, each bird was isolated for a few hours and its faeces collected and examined for trematode eggs which might indicate a background infection.

Metacercariae from fish were administered to birds in various ways. Where possible, the bird was isolated and left without food overnight, whole fish, or the appropriate organs being offered the next morning. If this was not eaten force feeding was sometimes necessary. Ducklings (which were generally infected within a few days of their arrival at the laboratory) tended to spread their food around the cage, eating little of it, in which case force feeding was required. To minimise stress a bird was returned to the others soon after ingesting metacercariae. A delay of 1 or 2 hours was necessary in the case of gulls since they tended to regurgitate recently swallowed food when handled (a habit which made force feeding difficult).

Strigeoid metacercariae from leeches and snails were also fed to ducklings to establish which species were

parasitising these invertebrate intermediate hosts.

Birds were foot-marked for identification using a chicken toe-punch.

C) Collection and treatment of adult worms.

Birds were killed by breaking the neck or by decapitation. The intestine was removed and cut into several pieces each of which was slit lengthwise and placed in a nylon tea-strainer (15 meshes to 1 cm) in a dish of 0.8% saline at 40°C.. The majority of adult worms passed through the tea-strainer and had accumulated on the bottom of the dish within an hour. When appropriate, the intestine was examined using a dissecting miroscope to check whether any worms remained.

Live adult worms were fixed either by rapid addition of hot 4% formol saline or heat killed by being passed over a bunsen flame while compressed under a cover-slip, and fixed by drawing formol saline under this. Fixed worms were stained with Gower's carmine for general morphology, and measurements taken from permanent, stained preparations.

In order to avoid measuring problems because of the irregular shapes of organs, all dimensions were taken from specimens mounted in a uniform position. Members of the family Strigeidae (genera Apatemon; Cotylurus) were not compressed on fixation and were mounted to give a lateral view of the body. Members of the family Diplostomatidae (genus Diplostomum) were mounted to give a dorsal or ventral view. Lengths of the forebody (and holdfast in diplostomatids) were taken along its long axis. Maximum lengths of the hindbody, ovary and testes were taken along or parallel to the long axis of the hindbody.

Maximum widths of parts of the body or its organs were measured perpendicular to the appropriate long axis. In members of the Strigeidae, suckers and pharynx were measured along their own axes.

Figures 3 and 4 illustrate the gross morphology of Apatemon (A.) gracilis (family Strigeidae) and Diplostomum (D.) gasterostei (family Diplostomatidae) and the nature of the dimensions recorded.

Identification of adult worms was by two means; a) by using keys in Dubois (1968a, 1970a), and b) by reference to their life-cycles, where known (Chapter 2).

RESULTS AND DISCUSSION

This section lists adult strigeoids obtained with notes on the history of each genus and species. The diagnosis of each is given in Appendix 4. Subgeneric names are only quoted where necessary.

Family Strigeidae Railliet, 1919

Szidat (1928) removed to new genera many forms previously placed in the genus Strigea Abildgaard, 1790 (see list of genera in Appendix 2). Adult specimens representing two of his genera were obtained during this study.

Genus Apatemon Szidat, 1928

Dubois and Pearson (1965) considered that the genus Apatemon should be divided into the subgenera Apatemon Szidat, 1928 and Australapatemon Sudarikov, 1959, members of the latter possessing a well developed genital cone, a structure rudimentary in the former. Cercariae of the subgenus Apatemon have 10 flame cells and develop

further in fish, while those of Australapatemon have 14 flame cells and penetrate leeches (see Appendix 4).

1) Apatemon (Apatemon) gracilis (Rudolphi, 1819) Szidat, 1928

Rudolphi applied the name Amphistoma gracilis to worms from the goosander (Mergus merganser L.) and the smew (M. albellus L.). Szidat (1931), working on the life-cycle of a form he called Apatemon gracilis, described a cercaria with 14 flame cells which encysted in leeches. His figure of the adult form (1929) shows a well developed genital cone and closely resembles Apatemon minor Yamaguti, 1933 from Japan. Dubois and Rausch (1948, 1950) considered that A. minor represented a subspecies of A. gracilis, and in 1953, Dubois listed 10 subspecies of A. gracilis, some with metacercariae in fish and some in leeches. Later Dubois (1968a p172) considered A. minor to constitute a valid species which he placed in the subgenus Australapatemon by virtue of its large genital cone. A. gracilis (= Amphistoma gracilis Rudolphi) he retained in the subgenus Apatemon. The form figured by Szidat (1929) as A. gracilis, Dubois attributed to A. minor. Both species occur in the Anatidae as adults, and have some hosts in common experimentally (list in Dubois, 1968a pp 152, 153 and 174).

Apatemon minor, having long been regarded a subspecies of A.gracilis, it is often not possible to be certain which species is referred to in the literature when A. gracilis is reported.

Table 1 summarises experimental attempts to obtain adult worms, and sources of the metacercariae used. In this study, all specimens of A. gracilis were obtained in the small intestines of ducklings fed metacercariae from

fish (3-spined sticklebacks, rainbow trout, and stone loach, see Chapters 2 and 5). The adult worm matures in about 4 days. It is depicted in Figures 3 and 5, and dimensions are given in Table 4. Dr. G. Dubois of Neuchâtel kindly confirmed the identity of worms sent to him which were derived from metacercariae in rainbow trout and in stone loach.

The only previous record of this species in Britain is that of Crocombe (1959, and quoted by Erasmus, 1962) who described Apatemon gracilis pellucidus Yamaguti, 1933, maturing in ducks fed metacercariae found encysted in the body cavity of the bullhead, Cottus gobio L.. This record was from South Wales.

2) Apatemon (Australapatemon) minor Yamaguti, 1933

Leeches at College Mill Trout Farm were seen to contain strigeoid metacercariae. When these were fed to ducklings, adult A. minor were obtained 4 days later from the first half of the small intestine. This species has frequently been recorded from British Anatidae (Williams, 1961).

Genus Cotylurus Szidat, 1928

Szidat established this genus to include strigeoids possessing a genital bulb. Odening (1969) proposed that the genus be divided into two subgenera, Cotylurus Szidat, 1928 and Icthyocotylurus Odening, 1969, the former with metacercariae in snails, and the latter with metacercariae in freshwater fish. If the criteria of Dubois (1970b) are followed, these two taxa should probably not be raised to generic rank as suggested by Niewiadomska (1971a).

3) Cotylurus (Cotylurus) cornutus (Rudolphi, 1808) Szidat, 1928

Adult worms of this species were obtained by feeding Lymnaea peregra from College Mill Trout Farm to ducklings. The snails contained tetracotyle larvae which matured to adult C. cornutus in the mid and hind regions of the small intestine of the ducklings. The life-cycle of C. cornutus in Scotland has been discussed by Williams, M.O. (1966a).

4) Cotylurus (Ichthycotylurus) variegatus (Creplin, 1825) Szidat, 1928; sensu Odening and Bockhardt, 1971

Metacercarial cysts found in the wall of the swim bladder, body cavity, and cranial cavity of 6 perch, Perca fluviatilis L., from Loch Lomond were fed to a black-headed gull. On autopsy 5 days later, 22 adult worms were found at the end of the small intestine, with a further five at the beginning of the large intestine. Most were ovigerous, up to 4 mm in length, with a terminal or slightly sub-terminal oral sucker and multi-lobed testes (see Figure 6). All belonged to the subgenus Ichthyocotylurus, and with the above set of characters could belong to C. pileatus (Rudolphi, 1802) Dubois, 1937 or C. platycephalus (Creplin, 1825) Szidat, 1928, or to C. variegatus (Creplin, 1825) Szidat, 1928 (= Cotylurus cumulitestis Dubois, 1962 according to Odening and Bockhardt, 1971). The material could not be convincingly be referred to any species on morphological grounds. However, it would seem likely that it belongs to C. variegatus for the following reasons.

a) The location of metacercariae in perch agrees with the data in Odening and Bockhardt (1971) for this species, whereas metacercariae of C. pileatus (i.e. Tetracotyle diminuta Hughes, according to Razmashkin,

1966) appear to localise around the heart (Hughes, 1928).

b) The distribution of the worms within the intestine agrees with that given by Odening and Bockhardt for C. variegatus whereas C. pileatus is recorded by Razmashkin (1966) as maturing within the small Intestine of gull chicks, and C. platycephalus within the cloaca, bursa, and large intestine (Dubois, 1968a p 226).

c) The ratio of forebody length to hindbody length of the present form is within the range given by Dubois (1968a p 207) for C. cumulitestis (= C. variegatus, see above) but not within that for C. pileatus (Dubois, 1968a p 220).

It should be pointed out that the status of C. variegatus is in dispute. Dubois (1974 in litt.) writes of some of the present forms sent to him for examination, that they "correspond assez bien au C. variegatus (Crepiin, 1825), tel que Odening et Bockhardt (1971) en decrit le cycle vital. ---- Mais la question reste de savior si C. variegatus est vraiment distinct de C.platycephalus (Crep. 1825), et sur ce point les opinions de Niewiadomska, d'Odening, et de Dubois ne trouvent pasun accord."

Family Diplostomatidae Poirier, 1886

Genus Diplostomum von Nordmann, 1832

Von Nordmann established this genus for the trematodes found by him in the eyes of freshwater fish. Diesing (1850) removed von Nordmann's Diplostomum clavatum into a new genus which he proposed to name Tylodelphys. Later both generic names, Diplostomum and Tylodelphys, were

given to adult genera (see general introduction, and Dubois, 1938 pp 161 and 298). Many authors still regard these as separate adult genera (e.g. Sudarikov, 1960a; Niewiadomska, 1973). Baer (1957) collected in Ivory Coast, an adult form he considered intermediate between Diplostomum and Tylodelphys. He proposed therefore to relegate these genera to subgeneric rank within Diplostomum von Nordmann. Dubois (1961) proposed Dolichorchis as a new subgenus, with Baer's species Diplostomum (Dolichorchis) marahoueense as the type. Dubois (1970a pp 274-276) included a further three subgenera in Diplostomum (see Appendix 2).

All adult Diplostomum spp. identified during this study belonged to the nominate subgenus.

5) Diplostomum (Diplostomum) spathaceum (Rudolphi, 1819) Braun, 1893

Based partly on the similarities of their cercariae, Dubois (1966, 1968b, 1970a pp 334-349) has reduced four species to subspecific status within D. spathaceum. These are, D. spathaceum huronense (La Rue, 1927) Hughes, 1929 and D. spathaceum indistinctum (Guberlet, 1923) Hughes, 1929 (=D.flexicaudum (Cort and Brooks, 1928) van Haitsma, 1931), both from North America; D. spathaceum murrayense (Johnston and Clelland, 1938) Johnston and Simpson, 1939 from Australia, and D. spathaceum spathaceum (Rudolphi, 1819) Braun, 1893 from Eurasia.

In the present study, adult D. spathaceum were obtained by experimental feeding of metacercariae to birds (see Table 2). The worms were only obtained from gulls and occurred in the small intestine. Dimensions of adult worms are given in Table 5, and an example

figured in Figure 7.

Dr. G. Dubois has confirmed the identity of adult D. spathaceum sent to him which were obtained from a black-headed gull fed eyes of roach from the Forth and Clyde Canal near Glasgow.

Diplostomum spathaceum has most recently been recorded from Britain by Sweeting (1971) and Betterton (1973). Williams, M.O. (1966a & b) has recorded the species from Scotland.

6) Diplostomum (Diplostomum) phoxini (Faust, 1918) Arvy and Buttner, 1954

Faust (1918) proposed the name Tetracotyle phoxini for a metacercaria described by Matare (1910) from the brains and cranial cavities of minnows (Phoxinus phoxinus L.) in Switzerland. Hughes (1929a) removed this form to the larval genus Diplostomulum. Arvy and Buttner (1954) in France, described the cercaria, metacercaria, and adult.

In the present study, metacercariae from the brains of minnows collected near Glasgow were fed to ducklings and to black-headed gulls. Adult worms were obtained from the intestine of both ducklings and gulls.

D. phoxini is widely distributed in Britain, metacercariae being found in minnows in Scotland and England (Ashworth and Bannerman, 1927) and in Wales (Rees, 1955). Rees (1955, 1957) made a careful study of the life-cycle of this species in Wales. Although adult worms have been raised experimentally in numerous hosts (see Dubois, 1970a p 326), they have been recorded in the wild only from the goosander (Mergus merganser L.) in Europe (Dubois, 1970a), and from the little grebe (Podiceps ruficollis (Pallas)) in Wales by Rees (1973).

7) Diplostomum (Diplostomum) gasterostei Williams, 1966

Berrie (1960b) demonstrated experimentally that two species of Diplostomum occurred as metacercariae in the eyes of the 3-spined stickleback (Gasterosteus aculeatus L.) in Scotland. One of these he recognised as D. spathaceum. The life-cycle of the other form was completed experimentally by Williams, M.O. (1966b). He proposed the name Diplostomum gasterostei for the species, of which adults matured experimentally in pigeons and ducklings. In the present study, adult worms were obtained experimentally from ducklings and black-headed gulls, and matured after 3 days in the first half of the small intestine. Table 3 summarises the feeding experiments with metacercariae of this species. The adult worm is depicted in Figures 4 and 8, and dimensions given in Table 6.

Adult specimens of D. gasterostei from black-headed gulls, and derived from cercariae from the Forth and Clyde Canal in one case, and from metacercariae in the eyes of perch from Loch Lomond in the other, were sent to Dr. G. Dubois, who confirmed their identity.

Although metacercariae in several species of British freshwater fish have been assigned to D. gasterostei (see Chapter 2), there have been no reports of adults since the original description. D. gasterostei has not been reported outside Britain.

8) Diplostomum (Diplostomum) sp.

Lenses of rainbow trout (Salmo gairdneri (Richardson)) infected experimentally with metacercariae of a Diplostomum sp. (see Chapter 2 p 94) were fed to four ducklings and to a black-headed gull. A single adult

worm was obtained in the small intestine of one duckling. It is shown in Figure 9, and its dimensions presented in Table 7.

No specific name can be attached to this form as yet. The single specimen was sent to Dr. G. Dubois, who considered it (in litt. 1974) to have affinities with Diplostomum mahonae Dubois, 1953 and with D. baeri Dubois, 1937.

GENERAL DISCUSSION

A detailed morphological discussion will not be included here. Rather, this brief discussion will be restricted to commenting on a few difficulties encountered in identifying adult strigeoids by their morphology, dimensions, and host specificity alone.

Diplostomum gasterostei will serve as an example. Groups of adults, raised in a single gull and derived from cercariae from the Forth and Clyde Canal, were either fixed in hot formol saline without compression (11 specimens) or were compressed lightly under a coverslip during fixation (20 specimens). Comparisons of a few dimensions (total body length, forebody length, hindbody length, and length of the posterior testis) using the student's t-test indicated no significant difference between groups treated either way. However, there were significant differences ($P < 0.05$) when these dimensions were compared between the above compressed worms and worms, similarily treated, from a gull fed perch eyes from Loch Lomond. Significant differences were also found ($P < 0.01$) when compressed adults from a duckling fed perch eyes from Loch Lomond were compared

with the groups above.

The significant size differences between adult D. gasterostei from ducklings and from gulls (fed metacercariae from Loch Lomond perch in each case) is not surprising in view of the observations of Berrie (1960a). More surprising is the significantly smaller size of adult worms derived from cercariae from the Forth and Clyde Canal than of worms derived from Loch Lomond perch eyes. Worms from both sources appeared to belong to D. gasterostei (according to the key in Dubois, 1970a p 292). When the life-cycles of both forms were completed to confirm their identities (Chapter 2), although their cercariae resembled one another, they did not have all their fish hosts in common. The question of subspecific taxa in strigeoids raised by these observations will be discussed further in Chapter 2.

As worms of a single species may reach different sizes in different bird hosts, it remains uncertain to what extent specimens of species (e.g. D. gasterostei and Diplostomum sp.) from as yet unknown definitive hosts will resemble specimens raised in laboratory hosts, and hence whether their affinities will be recognised.

Complications in interpreting feeding experiments became apparent during this study. Table 3 illustrates the variability in results when D. gasterostei metacercariae were fed to birds. Frequently worms obtained were non-ovigerous, and often none established at all. This was also occasionally observed in feeding experiments with D. spathaceum metacercariae. Campbell (1973) obtained similarily variable results after feeding metacercariae of Cotylurus flabelliformis

to numerous bird species. This serves to stress that negative results in feeding experiments should not be taken as proof of host specificity when only small numbers of host animals are used.

SUMMARY

During the present study, adult specimens of the following strigeoids were obtained by feeding their metacercariae to ducklings or to black-headed gulls.

1) Apatemon (Apatemon) gracilis (Rudolphi, 1819) Szidat, 1928
2) Apatemon (Australapatemon) minor Yamaguti, 1933
3) Cotylurus (Cotylurus) cornutus (Rudolphi, 1808) Szidat, 1928
4) Cotylurus (Ichthyocotylurus) variegatus (Creplin, 1825) Szidat, 1928; sensu Odening and Bockhardt, 1971
5) Diplostomum (Diplostomum) spathaceum (Rudolphi, 1819) Braun, 1893
6) Diplostomum (D.) phoxini (Faust, 1918) Arvy and Buttner, 1954
7) Diplostomum (D.) gasterostei Williams, 1966
8) Diplostomum (Diplostomum) sp.

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NOTES ON TABLES 1 TO 3

1) The sources of metacercariae used in these feeding experiments are discussed at greater length in Chapters 2 and 5.

2) Where eyes were fed to a bird, these were generally intact. Each table includes all experiments involving eyes for which examination of control eyes suggested the presence of the appropriate metacercaria. Where eyes contained metacercariae of more than one species, the feeding experiment appears in more than one table.

3) In some cases, fluke eggs from experimentally infected birds were collected, and ultimately yielded the appropriate cercariae in Lymnaea peregra. An asterisk (*) in the "source of metacercariae" column denotes cases where the life-cycle was continued experimentally from metacercaria to cercaria.

TABLE 1: Summary of feeding experiments to obtain adult Apatemon (Apatemon) spp. and sources of metacercariae used

Source of metacercariae	Birds used	Adult worms obtained and comments
* Rainbow trout from College Mill Trout Farm	10 Ducklings	Adult Apatemon gracilis were obtained from 9 of the 10 ducklings
	1 Gull	Gull negative
Rainbow trout experimentally infected with Apatemon cercariae from College Mill Trout Farm	5 Ducklings	Adult Apatemon gracilis from 4 of the 5 ducklings
Brown trout from College Mill Trout Farm	1 Gull	Negative
* Stone loach from River Almond, College Mill Trout Farm	2 Ducklings	Adult Apatemon gracilis from both ducklings
3-spined stickleback from River Almond	1 Duckling	Single adult Apatemon gracilis obtained
* 3-spined stickleback from Heidarvatn, Iceland	2 Ducklings	One duckling contained a single adult Apatemon gracilis
3-spined sticklebacks experimentally infected with Apatemon cercariae from Heidarvatn, Iceland	3 Ducklings	All negative
Eyes of Perch from Loch Lomond	3 Gulls	All negative
	1 Duckling	

TABLE 2: Summary of feeding experiments with metacercariae of *Diplostomum spathaceum*

Source of metacercariae	Birds used	Adult *D. spathaceum* obtained and comments
* Rainbow trout from College Mill Trout Farm	23 Ducklings 6 Gulls	Ducklings all negative 3 Gulls contained adult *D. spathaceum*
Rainbow trout experimentally infected with *D. spathaceum* cercariae derived from metacercariae in roach from the Forth and Clyde Canal (see below)	1 Gull	3 adult *D. spathaceum* obtained
Brown trout from College Mill Trout Farm	1 Duckling 1 Gull	Both negative
3-spined sticklebacks from College Mill Trout Farm	2 Ducklings	Both negative
* 3-spined sticklebacks from Forth and Clyde Canal	1 Duckling 1 Gull	Duckling negative Many adult *D. spathaceum* in Gull
* Roach from the Forth and Clyde Canal	3 Gulls	Two Gulls yielded many eggs but were not autopsied The remaining Gull contained adult *D. spathaceum*
* Perch from Loch Lomond	2 Ducklings 3 Gulls	Ducklings negative 1 Gull yielded many eggs and a single adult *D. spathaceum* on autopsy 4 weeks after infection: other Gulls negative

TABLE 3: Feeding experiments with metacercariae of *Diplostomum gasterostei*

Source of metacercariae	Birds used	Adult *D. gasterostei* obtained and comments
* Rainbow trout from College Mill Trout Farm	22 Ducklings 5 Gulls	7 Ducklings contained *D. gasterostei* All Gulls negative
Rainbow trout experimentally infected with *D. gasterostei* cercariae from *L. peregra* from the Forth & Clyde Canal	1 Duckling 1 Gull	Duckling negative Many adult worms in Gull
Brown trout from College Mill Trout Farm	1 Duckling 1 Gull	Both negative
* 3-spined sticklebacks from the Forth & Clyde Canal	1 Duckling 1 Gull	Duckling negative Many adult worms in Gull
3-spined sticklebacks experimentally infected with *D. gasterostei* cercariae from the Forth & Clyde Canal	1 Gull	Many adult worms obtained
3-spined sticklebacks experimentally infected with *D. gasterostei* cercariae from Packington, Warwickshire	1 Duckling	Many adult worms obtained
* Perch from Loch Lomond	3 Ducklings 3 Gulls	All Ducklings contained adult worms All Gulls contained adult worms

TABLE 4: Dimensions (range and mean, in μm) of adult Apatemon gracilis

		Adults raised in ducks from metacercariae in stone loach from College Mill Trout Farm	Adults raised in ducks from metacercariae in sticklebacks from College Mill Trout Farm	Adults raised in ducks from metacercariae in trout from College Mill Trout Farm
Total Body	Length	1275 - 1449 (1386)	970	1031 - 1567 (1200)
Forebody	Length	480 - 612 (522)	357	395 - 697 (479)
	Width	325 - 372 (346)	248	308 - 365 (341)
Hindbody	Length	782 - 930 (864)	613	620 - 870 (722)
	Width	287 - 395 (327)	209	266 - 356 (292)
Oral Sucker	Length	97 - 105 (102)	97	84 - 97 (93)
	Width	76 - 89 (83)	67	68 - 89 (81)
Pharynx	Length	49 - 61 (56)	44	48 - 58 (54)
	Width	44 - 65 (56)	49	40 - 49 (45)
Ventral Sucker	Length	124 - 148 (137)	110	103 - 120 (112)
	Width	105 - 148 (120)	97	89 - 133 (107)
Ovary	Length	80 - 120 (97)	68	72 - 95 (86)
1st Testis	Length	190 - 228 (207)	97	171 - 190 (180)
2nd Testis	Length	205 - 310 (260)	146	193 - 262 (223)
Dimensions of Eggs		92-102 (97)/65-74 (70)	88 (length)	
No. Specimens		(9)	(1)	(4)

TABLE 5: Dimensions (range and mean, in μm) of adult Diplostomum spathaceum from a gull fed metacercariae from canal roach

10 specimens

Total Body	Length	1390	-	2040	(1740)
Forebody	Length	845	-	1185	(996)
	Width	353	-	468	(391)
Hindbody	Length	580	-	1010	(820)
	Width	189	-	328	(265)
Oral Sucker	Length	20	-	24	(23)
	Width	19	-	26	(21)
Pharynx	Length	16	-	19	(18)
	Width	10	-	13	(11)
Ventral Sucker	Length	23	-	30	(25)
	Width	19	-	32	(24)
Holdfast	Length	54	-	87	(69)
	Width	33	-	73	(48)
Ovary	Length	19	-	30	(26)
	Width	23	-	39	(29)
1st Testis	Length	38	-	71	(54)
	Width	52	-	98	(78)
2nd Testis	Length	54	-	101	(71)
	Width	56	-	81	(69)

TABLE 6: Dimensions (range and mean, in μm) of adult D. gasterostei from various sources

Dimensions		Adults from metacercariae experimentally derived from Forth & Clyde Canal cercariae, raised in a gull, compressed on fixation	Adults from metacercariae experimentally derived from Forth & Clyde Canal cercariae, raised in a gull, uncompressed on fixation	Adults from metacercariae in Loch Lomond perch, raised in a gull, compressed in fixation	Adults from metacercariae in Loch Lomond perch, raised in a duck
Total Body	Length	1170 - 1638 (1420)	1160 - 1690 (1389)	1038 - 1380 (1214)	780 - 995 (870)
Forebody	Length	574 - 868 (728)	535 - 970 (726)	481 - 689 (570)	341 - 504 (457)
	Width	535 - 736 (644)	310 - 426 (381)	364 - 612 (481)	318 - 504 (450)
Hindbody	Length	636 - 938 (800)	644 - 806 (725)	534 - 713 (634)	341 - 520 (442)
	Width	372 - 488 (418)	217 - 279 (253)	271 - 325 (303)	333 - 403 (372)
Oral Sucker	Length	65 - 80 (72)	55 - 74 (67)	67 - 78 (71)	57 - 67 (61)
	Width	67 - 86 (76)	57 - 70 (62)	61 - 76 (68)	57 - 67 (61)
Pharynx	Length	61 - 82 (68)	55 - 70 (62)	53 - 65 (59)	63 - 67 (65)
	Width	32 - 51 (42)	38 - 46 (42)	32 - 38 (35)	19 - 34 (29)
Ventral Sucker	Length	72 - 95 (82)	68 - 86 (78)	67 - 84 (71)	55 - 61 (57)
	Width	86 - 108 (101)	70 - 82 (77)	80 - 105 (92)	67 - 82 (76)
Holdfast	Length	124 - 190 (162)	171 - 228 (200)	141 - 190 (172)	104 - 133 (116)
	Width	105 - 186 (145)	133 - 179 (160)	108 - 171 (132)	105 - 129 (114)
Ovary	Length	86 - 133 (101)	78 - 116 (94)	67 - 93 (78)	67 - 89 (76)
	Width	123 - 175 (144)	80 - 116 (91)	88 - 135 (115)	87 - 143 (112)
1st Testis	Length	209 - 304 (243)	167 - 262 (218)	137 - 246 (174)	103 - 137 (118)
	Width	198 - 357 (274)	133 - 198 (159)	201 - 270 (234)	137 - 198 (171)
2nd Testis	Length	213 - 342 (285)	205 - 330 (267)	163 - 228 (192)	84 - 160 (122)
	Width	315 - 399 (357)	171 - 247 (208)	209 - 308 (264)	236 - 350 (312)
No. Specimens		(20)	(11)	(10)	(5)

TABLE 7: Dimensions (in μm) of adult of *Diplostomum* sp.

	Length	Width
Total Body	752	
Forebody	380	319
Hindbody	350	323
Oral Sucker	61	49
Pharynx	51	21
Ventral Sucker	42	78
	Holdfast not measured	
Ovary	61	121
1st Testis	101	176
2nd Testis	131	262

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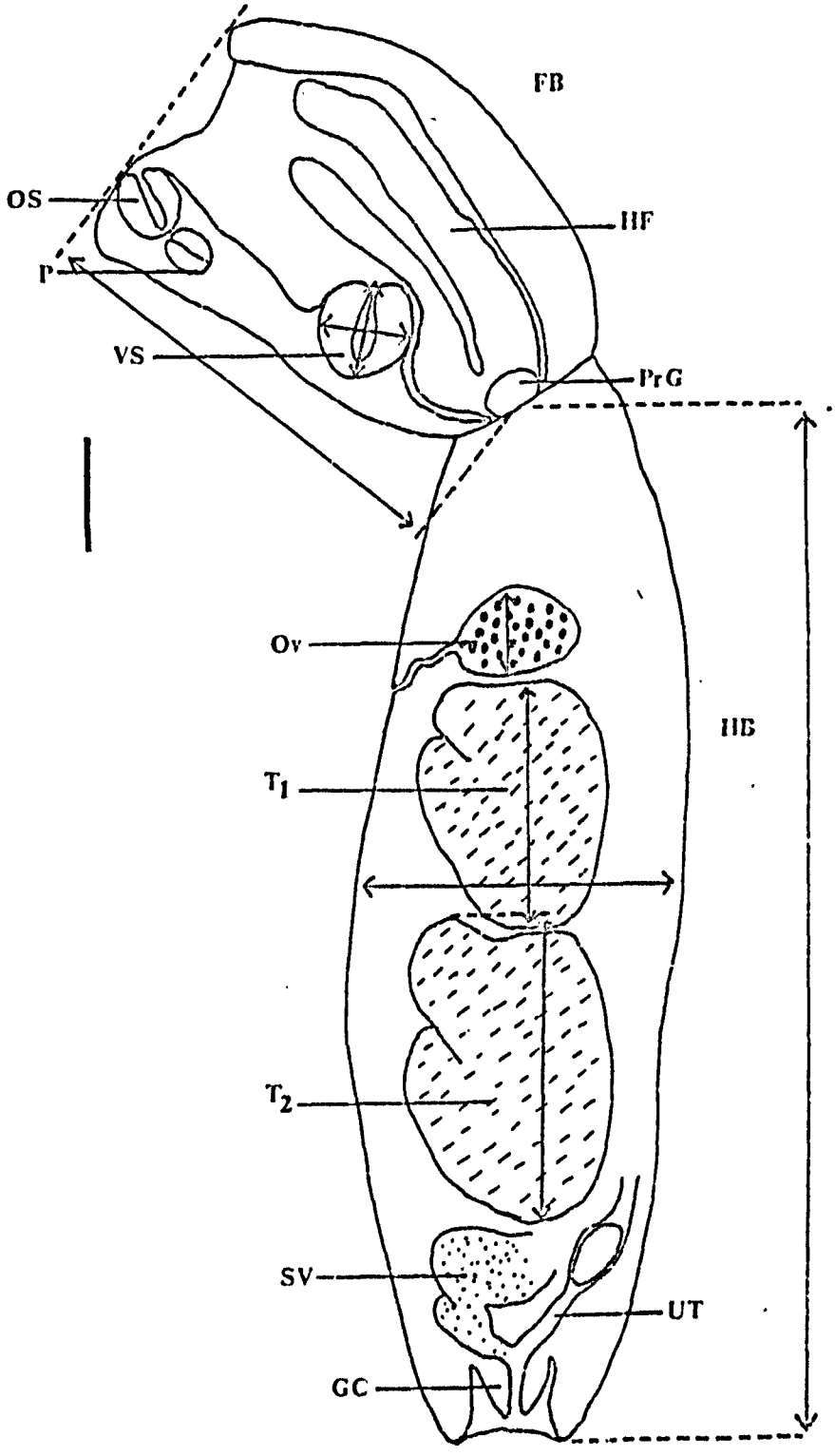
Figure 3 Diagram of the gross morphology of an adult Apatemon (A.) gracilis in lateral view. An indication is given of some of the dimensions measured. (Note; total body length obtained by summing axial lengths of forebody and hindbody) Bar represents approximately 100μm.

key to abbreviations on page xi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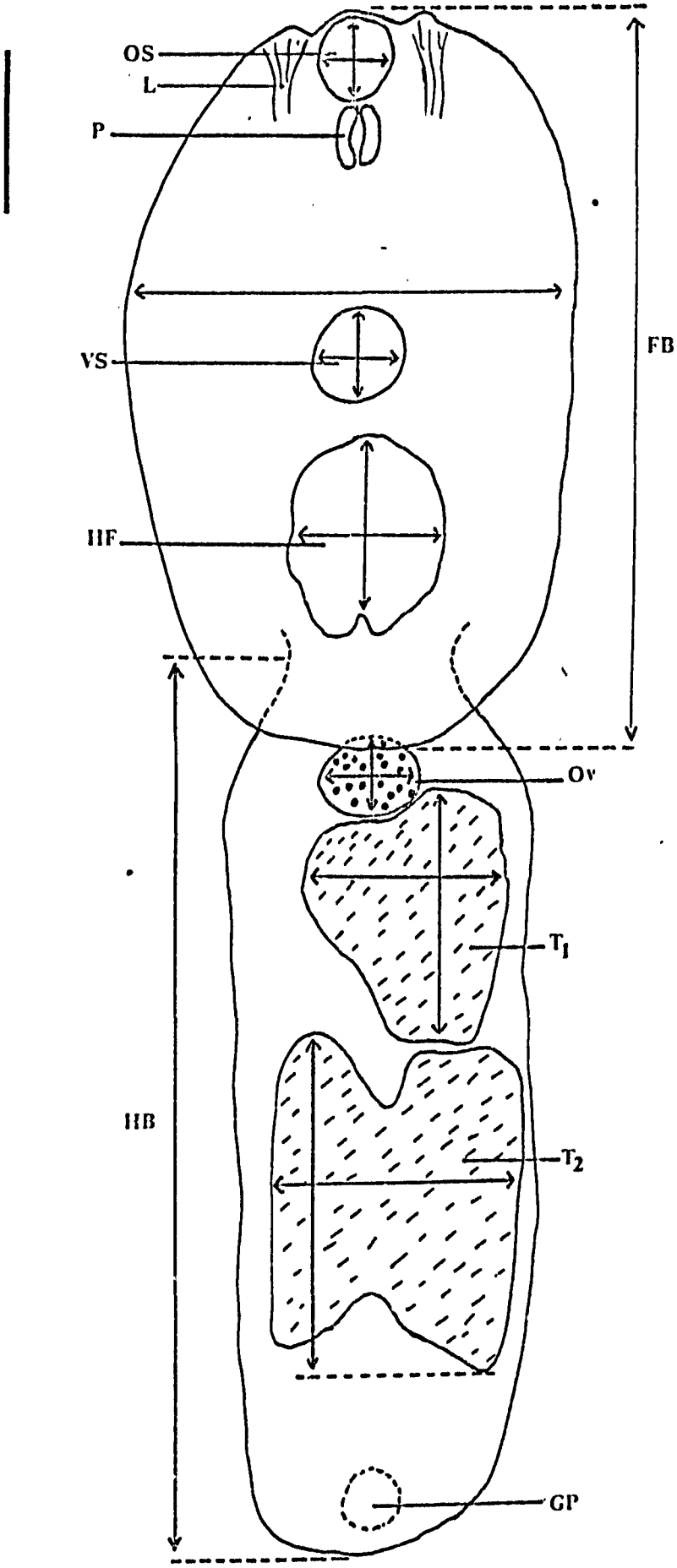
facing page 33

Figure 4 Diagram of the gross morphology of an adult Diplostomum (D.) gasterostei in ventral view. An indication is given of some of the dimensions measured. The bar represents approximately 100μm

key to abbreviations on page xi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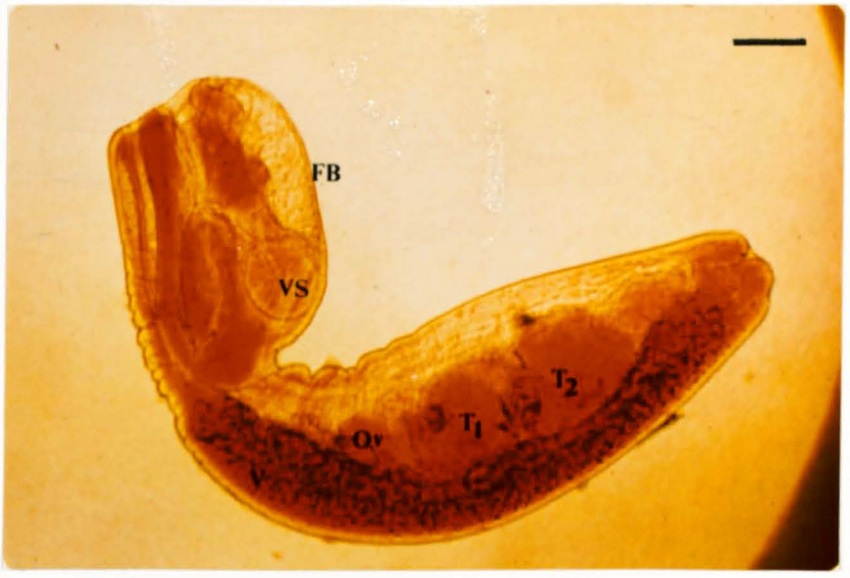
facing page 34

Figure 5 Adult Apatemon gracilis from duckling fed metacercariae from stone loach. Lateral view. Fixed in hot formol saline, stained with Gower's carmine. Bar represents approximately 100μm.

Figure 6 Adult Cotylurus variegatus from black-headed gull fed metacercariae from perch. Lateral view. Fixed in hot formol saline, stained with Gower's carmine. Bar represents approximately 500μm.

key to abbreviations on page xi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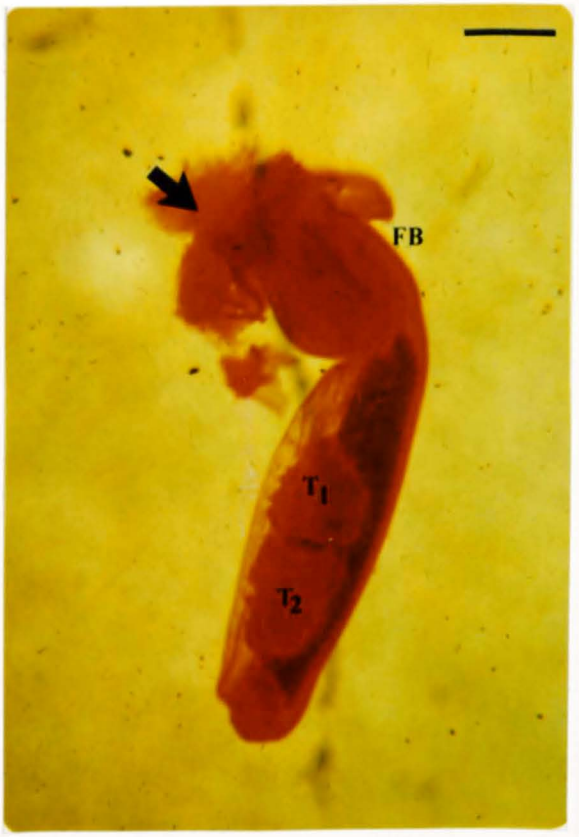
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Figure 7 Adult Diplostomum spathaceum from black-headed gull fed metacercariae from roach. Ventral view. Fixed in hot formol saline, stained in Gower's carmine. Bar represents approximately 250μm.

Figure 8 Adult Diplostomum gasterostei from gull fed metacercariae from perch. Ventral view. Fixed in hot formol saline, stained with Gower's carmine. Bar represents approximately 250μm.

Figure 9 Adult Diplostomum sp. from duckling fed lenses from rainbow trout. Ventral view. Fixed in hot formol saline, stained with Gower's carmine. Bar represents approximately 100μm.

key to abbreviations on page xi


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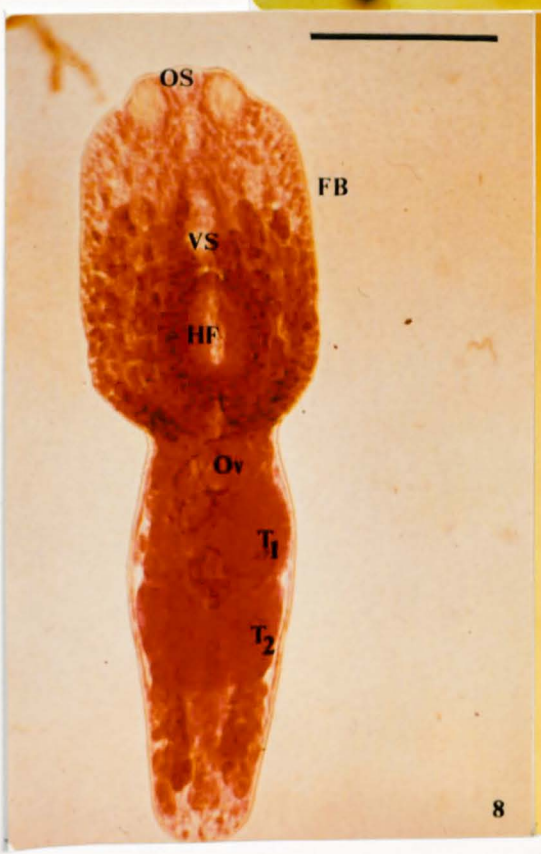
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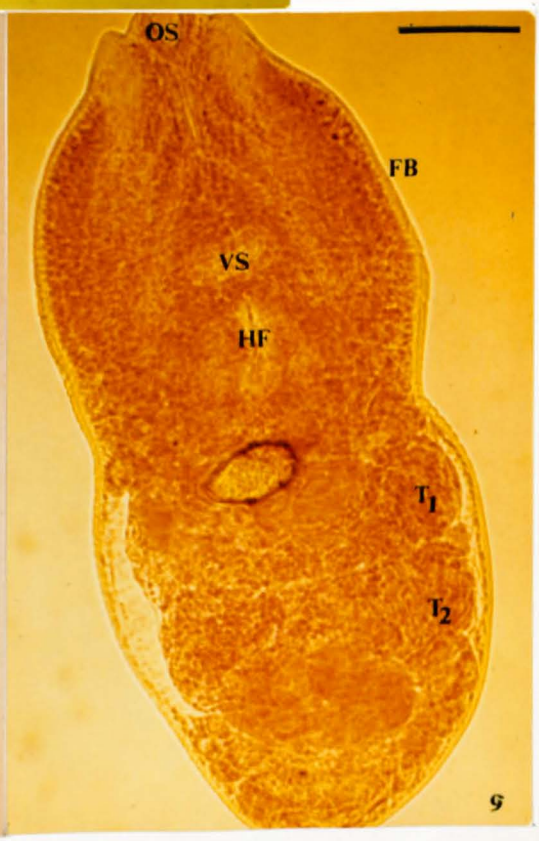
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CHAPTER 2 COMPLETION OF THE LIFE-CYCLES.

PREFACE

Chapter 1 has listed and given the history of those strigeoids obtained as adults. The purpose of Chapter 2 is to report work done on the life-cycles of the worms, paying special attention to their cercariae and metacercariae. The chapter is presented in two sections. The first is a detailed examination of the taxonomy of the cercariae with a view to establishing diagnostic criteria. The second section includes experimental data on the susceptibility of fish to different cercariae, and aspects of the biology of the metacercariae.

A summary of the chapter may be found on page 110.

SECTION 1 THE CERCARIAE

INTRODUCTION

Strigeoid cercariae obtained from the field during this study were frequently used as the starting point in life-cycle studies. It soon became apparent that they present more features lending themselves to taxonomic use than do metacercariae. Spination, caudal structures, penetration glands, and the excretory system are features either lost subsequently, or greatly modified, the simpler cercarial arrangement being obscured. To this list may be added behavioural and physiological features unique to the cercariae, including swimming behaviour, resting postures, and specificity with respect to the next host. Since all trematode life-cycles have in common a molluscan host, no matter how diverse their final hosts, the accurate identification of cercariae is of considerable value in analysing a trematode fauna, especially where few molluscs occur (as at College Mill Trout Farm, see page 180).

By the early years of this century, many cercariae were described, but few complete life-cycles known. Thus the system of Luhe (1909) for classifying cercariae relied on superficial morphological criteria, notably the shape of the tail and the arrangement of suckers. A major step towards a more natural classification came with the recognition of the importance of the excretory system. Cort (1917), describing the excretory system of six furcocercariae, proposed that his "observations indicate the conservativeness of the excretory system in trematodes and its value in establishing relationships in this group". His ideas were rapidly extended by Faust (1919, 1924, 1932)

who proposed that the cercarial excretory system could be represented by a "flame cell formula", and by Sewell (1922) and Miller (1926). La Rue (1957) used features of the cercarial excretory system as a primary criterion in his classification of the Digenea. Following the work of Lutz (1921), strigeoid cercariae were recognised to be furcocercariae possessing long tail furcae and a rather simple excretory system.

Cercariae in the Strigeidae and Diplostomatidae may be readily placed in appropriate adult genera or subgenera according to their excretory systems and the number and arrangement of their penetration glands (Niewiadomska, 1970, 1971b). Many cercariae for which the adults are unknown may be referred to the correct genus by comparison with cercariae of known adult species. However, criteria for separating species within a genus or subgenus using cercarial characters have never satisfactorily been established and authors have emphasised many differing features as diagnostic criteria. As discussed by Faust (1919) and Sewell (1922), the flame cell formula is of little value in separating closely related species. Niewiadomska (1971) carried out the most substantial study to date on diagnostic criteria in furcocercariae. She considers that body proportions, caudal body numbers and details of spination may afford diagnostic criteria, with cercarial resting posture a less important character.

Most authors are agreed that descriptions should be based on specimens freshly emerged from a mollusc, since pre-emergence cercariae may possess structures not seen subsequently (Brackett, 1939), and degenerative changes may occur, especially in the caudal bodies of the tail-stem,

after some hours of free swimming life (Erasmus, 1958). However, there is less uniformity in the literature concerning the preparation of cercariae for measurement. Dubois (1929) discussed the manner in which dimensions of cercariae vary according to the fixative used. In this context, the observations of Cort and Brackett (1937a) are worth quoting in full. " It is a difficult matter to make measurements that can be used for specific determination for a species of cercaria. The great power of extension and contraction makes measurements taken on living material entirely too variable. For the strigeid cercariae, measurements on heat killed specimens showed too great variation. After trying several methods of killing, we found that cercariae fixed in hot formalin gave the most constant results." In most recent cercarial descriptions, authors have taken measurements from specimens fixed in hot 10% formalin, an approach also recommended by Nasir and Erasmus (1964) in their key to British cercariae.

The value of dimensions in cercarial taxonomy has often been questioned. Wesenberg-Lund (1934 p 6) was of the opinion that dimensions could only be used as a general guide. More recently, Niewiadomska (1964a) commented on the varying sizes of cercariae of Codonocephalus urniger (Rud.) Luhe, 1909 from different localities, and Donges (1964) showed that cercariae of Posthodiplostomum cuticola (Nord.) Dubois, 1936 developing in snails at 15°C were significantly larger than those developing at 24°C. The same author (Donges, 1974) observed that samples of the furcocercaria of Euclinostomum heterostomum (Rudolphi, 1809) from different intermediate host individuals may differ significantly in measurements.

The arrangement of spines on cercariae has been described by many authors. Cercariae in the Strigeidae and Diplostomatidae possess a collar of spines posterior to the mouth, and frequently an apical tuft anterior to the mouth. the rest of the body may carry spines scattered randomly or arranged in rings. The ventral sucker is armed with rings of spines and the tail-stem and furcae may carry small spines. Many descriptions, early and recent, are inadequate with respect to spination. However, the value of spine arrangements, especially of those on the ventral sucker, was recognised in the 1920's when Cort and Brooks (1928) and Dubois (1929) included details of these in species diagnoses. More recently, Pearson (1956) reported that cercariae of the closely related Alaria canis La Rue and Fallis, 1934 (= Alaria marcianae (La Rue, 1917) Walton, 1949) and A. arisaemoides Augustine and Uribe, 1927 differ in their ventral sucker armament. Niewiadomska (1971b) separated cercariae of three species of Cotylurus on the basis of body and ventral sucker spination.

Other tegumentary structures which have been used in species separation are the presence or absence of a furcal "fin-fold", and the arrangement of sensory papillae on the body, tail-stem, and furcae. Several cercariae referable to the subgenus Diplostomum possess a fin-fold on the margins of the furcae. These include Cercaria helvetica XV Dubois, 1929 and the cercaria of Diplostomum indistinctum (= D. spathaceum, see p 19) described by Shigin (1968a). Williams (1966b) used this character to separate the cercaria of D. gasterostei from Cercaria helvetica XV. Papillae occur on the surface of trematodes at sensory nerve endings (Dixon and Mercer, 1965). Several types

have been described (Matricon-Gondran, 1971) and may be demonstrated by the silver nitrate impregnation method of Wagner (1961). Possibly more than one type of papilla occurs on strigeoid cercariae, although this has yet to be confirmed. Bibby and Rees (1971) described sensory papillae, each bearing a long cilium, on the tail-stem of cercariae of D. phoxini. Papillae on the body possess a much shorter cilium. The most extensive study to date of the distribution of sensory papillae on cercariae is that of Richard (1971) who considered the subgenus Diplostomum very homogeneous in this respect.

Niewiadomska (1971b) considered that cercarial resting posture may have systematic significance. This character has been given a fundamental position in the keys devised by Dubois (1970a p 278 et seq.) for cercariae of the subgenus Diplostomum, and is frequently used in specific diagnoses. Cort and Brackett (1937b) demonstrated that several strigeoid cercariae in Michigan may be tentatively identified on the basis of their resting posture and swimming behaviour. The latter has received scant attention from systematists. Studies by Haas (1969) on responses of D. spathaceum cercariae to light and mechanical stimuli, and by Donges (1963, 1964) on Posthodiplostomum cuticola were very detailed, but were not discussed comparatively.

Another important character in Dubois' (1970a) key for Diplostomum cercariae is the number of caudal bodies in the tail-stem. This appears very constant within a species. The cercaria of D. phoxini, for example, possesses six pairs of large caudal bodies (Arvy and Buttner, 1954) and that of D. spathaceum possesses numerous pairs (over 20). Similarily, the genus Cotylurus includes species of which

the cercariae possess caudal bodies and species which lack these altogether.

Dubois (1968a p 140), in his key for cercariae of the subgenus Australapatemon, attached importance to the exact position of the penetration glands (i.e. whether paracetabular or postacetabular). Several authors have demonstrated that the position of the penetration glands relative to the ventral sucker may alter with the movements of the cercaria. Pearson (1956, 1961) figured such variation in cercariae of Alaria arisaemoides and Neodiplostomum (=Fibricola) intermedium (Pearson, 1959). Odening and Bockhardt (1971) recorded considerable variation in the position of the anterior pair of penetration glands in Cotylurus variegatus cercariae.

As a secondary character in his key for Australapatemon cercariae, Dubois (1968a p 140) used the presence or absence of excretory commissures and the degree of development of these. The inconstancy of this character has been demonstrated several times. Johnston and Beckwith (1947a) figured the cercaria of Apatemon (Australapatemon)intermedius (Johnston, 1904) as possessing both a pre- and a postacetabular commissure, or only one of these, the other being represented by blind ending ducts. Niewiadomska (1971b) has recorded a similar variation in Apatemon cercariae from a single snail. In some of her specimens, neither commissure was complete, both being represented by blind ending ducts. A similar variation was noted during this study in cercariae of an Apatemon (Australapatemon) sp.emerging from a single Lymnaea peregra from Iceland.

Another feature of the excretory system to which

significance has been attached is the "Island of Cort". During cercarial development, the two excretory ducts running down the embryonic tail-stem fuse to form a single duct. This fusion may be incomplete proximally leaving a tiny "island" immediately posterior to the bladder and bounded by the excretory ducts (Komiya, 1938). Although used in the past as a diagnostic aid, less reference has been made to this structure in recent years. The island is difficult to see, and no information is available as to its intra-specific variation.

Dubois (1968a) used the point of bifurcation of the oesophagus relative to the ventral sucker as a primary character in separating cercariae of Cotylurus spp. Nasir (1962) also considered this feature of value, adding the topography of the caeca as an additional character. Nothing has been published to throw doubt on the usefulness of these characters, although it is noteworthy that Niewiadomska (1971b) considered gut shape subordinate to spination as a means of separating Cotylurus spp.

Irregular yellow patches lying in front of the ventral sucker have been described for several cercariae in the subgenus Diplostomum. Dubois (1970a pp 280-1) synonymised Cercaria X Taylor and Baylis, 1930, Cercaria chromatophora Brown, 1931 and Cercaria paracauda Iles, 1959 partly on the basis of their all possessing yellow patches. Erasmus (1958) has demonstrated that the distribution of glycogen in Cercaria X coincides with these patches.

MATERIALS AND METHODS

A) Experimental infection of snails and sources of cercariae

The snail species used exclusively was Lymnaea peregra (Muller). Snails were maintained in shallow polythene containers (60 by 30 by 15 cm deep) with a slow inflow of water at one end and a screened overflow at the other. The bottom of all tanks were covered with chicken grit containing fragments of sea shells to provide calcium. Snails were fed with fresh lettuce.

Initially, adult L. peregra were collected from the Forth and Clyde Canal close to Glasgow. After some weeks in the laboratory, they had deposited numerous egg masses in their tanks. The adult snails were then removed, allowing the egg masses to develop and the young snails to hatch. Only these parasite-free laboratory reared snails were used in infection experiments.

In experimental infections of snails, fluke eggs were collected as indicated on page 151. These were allowed to develop at about 25°C until shortly before hatching. The embryonated eggs were then transferred in a dish to a tank containing snails. Infections were usually carried out at between 15 and 20°C.

Experimentally infected snails, or snails collected from the field during summer months (by hand or using a dip-net) were placed in individual 7.5 by 2.5 cm glass tubes, and left in daylight or under a lamp for some hours. Cercariae emerging could be removed with a drawn-out Pasteur pipette, or concentrated by pouring through a sintered glass Gooch crucible (porosity 2).

B) Treatment of the cercariae.

Cercariae killed in hot 10% formalin were measured in this medium, coverslip pressure on the specimens being avoided. Hanging posture and swimming behaviour were observed using a dissecting microscope.

To demonstrate most structures, live cercariae were examined in water under a coverslip. As the water evaporated or was drawn off with filter paper, internal features, notably the excretory system, became more apparent in the compressed specimen. A high power objective lens (times 100) with phase-contrast illumination was used in studying fine details of cercarial anatomy. With increasing compression, cercariae would frequently rupture, tegumentary spines being most easily seen after this. Neutral red was occasionally used as a vital stain to demonstrate gut caeca and penetration glands.

To stain tegumentary papillae, a modification of the method of Sweeting (1971 p 104) was used. Cercariae in a solid watch-glass were cooled to 4°c in a refrigerator at which temperature they were almost inactive. Supernatant water was then removed and a cold 0.5% solution of silver nitrate poured in. The use of cold media gave the best staining and minimised contraction and distortion of the cercariae. Specimens were left in silver nitrate in the dark (temperature no longer important) for from several minutes to several hours. After 3-4 careful rinses with water, cercariae were placed on a slide in a drop of 10% glycerol in 70% alcohol. Excess medium was withdrawn from under the coverslip using filter paper, and the coverslip ringed with Canada balsam. Papillae could best be seen using a times-100 objective and bright-field illumination.

After some minutes in bright light, each papilla was visible as a dark brown ring. Openings of the penetration gland ducts and the excretory ducts also stained in this way. The course of sensory nerves was often marked by a line of brown precipitate after a long incubation in silver nitrate.

Cercariae of several species were examined in a scanning electron microscope. Live cercariae in a tube of water were chilled until they settled to the bottom. The supernatant water was removed and hot 10% formalin added rapidly to kill and fix the cercariae. This method of killing yielded some cercariae in which the ventral sucker was everted, and its spines the more easily seen. The cercariae were post-fixed in 0.5% osmium tetroxide and subsequently rinsed in distilled water. They were then pipetted onto a 12 mm diameter circular coverslip and freeze-dried. The coverslip was fixed to a specimen stub which was placed in a Cambridge 600 scanning electron microscope, and examined with accelerating voltages of 7.5 or 15 Kv.

RESULTS

Strigeoid cercariae of eight species were obtained during this study and identified as far as possible. They were of *Apatemon gracilis*, *A. minor*, *Cotylurus cornutus*, *Diplostomum spathaceum*, *D. phoxini*, *D. gasterostei*, *Diplostomum* sp., and *Diplostomum* (*Tylodelphys*) sp.

Only those cercariae penetrating fish were studied in detail, the remainder being included for completeness.

Apatemon (A.) gracilis;

Cercariae of this species emerged from naturally infected Lymnaea peregra collected at College Mill Trout Farm, and from Heidarvatn, a lake in southern Iceland (see Chapter 5).

Starting from metacercariae in fish, the life-cycle was completed experimentally on several occasions, thus yielding cercariae of known ancestry (metacercariae used came from stone loach (Noemacheilus barbatulus (L.)) and from rainbow trout (Salmo gairdneri Richardson) both collected at College Mill Trout Farm, and from 3-spined sticklebacks (Gasterosteus aculeatus L.) from Heidarvatn).

The cercaria of Apatemon gracilis has been described previously by Vojtek (1964). Descriptions of a number of similar cercariae are found in the literature, and will be discussed later. The present description is based largely on cercariae emerging from several Lymnaea peregra collected on 31 July 1973 at College Mill Trout Farm.

The dimensions (range and mean) of 10 specimens are as follows;

body length	110 - 135μm	(123μm)
maximum body width	32 - 40	(35)
tail-stem length	124 - 142	(137)
tail-stem width	32 - 44	(38)
furca length	154 - 163	(159)
anterior organ length	30 - 34	(31)
anterior organ width	21 - 25	(23)
ventral sucker length	15 - 17	(16)
ventral sucker width	15 - 17	(16)

The cercaria is an active swimmer, rarely pausing, and then generally for less than one second. It swims tail

first by a strong lateral flexing of the tail-stem, the furcae being used as oars, and the body held extended.

The general morphology of the cercaria is shown in Figure 10.

The anterior organ is large, and through it pass the prepharynx and the penetration gland ducts. At least over its posterior and lateral surface, the anterior organ has a thin cortex of circular muscle, the anteriormost extent of which could not be determined. Muscle fibres originating at the prepharynx close behind the mouth radiate outwards and slightly posteriorly to the margin of the anterior organ. A few fibres also run longitudinally to the posterior end of this organ.

The mouth opening is slightly subterminal ventrally, and the prepharynx is at its broadest immediately behind this. For the rest of its length the prepharynx is narrow. From the movement of refractile material within it and the way in which its contents are often contained within a central longitudinal helix, it appears to be muscular in nature. A small pharynx lies a short distance behind the anterior organ. The short oesophagus opens almost immediately into two short, broad caeca which terminate well forward of the ventral sucker.

There are two unpigmented eyespots lateral and slightly anterior to the ventral sucker.

The granular penetration gland cells occupy much of the body lateral and posterior to the ventral sucker. Although distinct boundaries between the cells are not apparent, six pairs of large clear nuclei, each with a dark central region, are clearly visible within the glandular mass. However, no more than four ducts emerging

on each side from the gland cells could be counted. The gland cell ducts run forward, are thrown into a loop at the level of the pharynx if the body is contracted, and are often dilated within the anterior organ. The duct bundles open to the outside anterior to the mouth on each side.

Figure 11 shows some features of the cercarial body.

The tail-stem contains six pairs of large caudal bodies, each with a clear nucleus, and arranged, in ventral view, regularily along the tail-stem on either side of the excretory canal. The caudal bodies are anchored to the excretory canal rather than to the wall of the tail-stem.

The excretory system consists of ten flame cells, four each side in the body, and one in the tail-stem. There is a postacetabular commissure. An anterior collecting duct on each side of the body drains the two flame cells anteriorly in the body. Similarily, a posterior collecting duct drains the caudal flame cell and the two lying posteriorly in the body. The collecting ducts on each side unite laterally behind the ventral sucker, their common duct being thrown into several tight loops before joining the bladder arm. The bladder is Y-shaped. The bladder arm arising dorsally on each side runs forwards and laterally to a point behind the ventral sucker where it meets the common collecting duct and the postacetabular commissure. The flame cell formula is thus $2[(2)+((2)+(1))]= 10$. The excretory canal passes along the axis of the tail-stem and bifurcates distally. Its branches open halfway along the dorsal margin of each furca, immediately after a slight dilation of the duct.

The anterior organ bears an apical tuft of spines in a crescent anterior to the mouth, their hooked ends pointing towards the mouth opening. In seven specimens, the apical tuft consisted of 7 spines (4 specimens), 8 spines (2), and 10 spines (1). The largest of the spines is 3.5μm in length. A post-oral collar of posteriorly directed spines in six or seven regular and closely spaced transverse rows, rings the body just behind the mouth. Posterior to this, and evenly distributed back to the level of the caeca, the body carries a sparse covering of small spines (Figure 19). The ventral sucker bears three irregular concentric rings of large inwardly directed spines, each about 3.0μm long. There are 18-20 spines in each ring. Immediately behind this sucker is a small group of posteriorly directed spines (Figure 24). Four bands of small spines run along the tail-stem, one either side of the midline, dorsally and ventrally, and continue along the margins of the furcae. Each band consists of 2-4 rows of spines.

The arrangement of sensory papillae as revealed by silver nitrate staining is shown in Figures 30 and 32. Papillae are concentrated anteriorly and ventrally on the body. The presence of a cilium is difficult to determine on body papillae. However, the papillae on the tail-stem each bear a cilium as long as the tail-stem is wide, and those on the furcae each bear a short cilium. These are best seen in live specimens using phase-contrast illumination. At the tip of each furca, two small cilia arise from what appears to be a single papilla. No variation was observed in the number or arrangement of papillae on the body or the furcae. The papillae on the tail-stem, especially those proximally, were less constant in their distribution.

The tail-stem is thrown into numerous transverse ridges by the presence of superficial circular muscles. Two bundles of longitudinal muscles ventrally, and two dorsally, pass down the tail-stem and into the furcae close to the furcal margins. Fine dorso-ventral muscle fibres, running from margin to margin, lie under each face of the furcae. The central lumen, occupying much of each furca and the tail-stem, appears devoid of muscle cells.

Apatemon (Australapatemon) minor;

Cercariae referable to this species emerged from Lymnaea peregra from Iceland (see Chapter 5), from College Mill Trout Farm, and from the Forth and Clyde Canal close to Glasgow. Nothing need be added to the description of this cercaria by Iles (1959). Silver nitrate staining of specimens from the Forth and Clyde Canal revealed a pattern of sensory papillae very different from that observed in Apatemon gracilis (Figure 33).

Cotylurus (C.) cornutus;

Cercariae of C. cornutus emerged from Lymnaea peregra from Iceland (see Chapter 5), and from College Mill Trout Farm. The cercaria has been described by Dubois (1929).

Diplostomum (D.) spathaceum;

Lymnaea peregra shedding cercariae of this species were collected from College Mill Trout Farm, from the Forth and Clyde Canal near Glasgow, and from gravel-pits at Packington, Warwickshire.

The life-cycle of Diplostomum spathaceum was completed experimentally on several occasions. Metacercariae from roach and from 3-spined sticklebacks (both from the Forth and Clyde Canal), from perch (Loch Lomond), and from rainbow trout (College Mill Trout Farm) were fed to separate black-headed gulls. Cercariae were obtained subsequently from L. peregra exposed to eggs from the faeces of these birds (see also Table 2 and page 19).

Sweeting (1971 p 102) has briefly described this cercaria from the Leeds-Liverpool Canal in England. No previous British description has been found, nor is it included in the key to British freshwater cercariae (Nasir and Erasmus, 1964). The present description is based largely on specimens obtained experimentally from some of the above sources.

The cercaria of D. spathaceum swims tail first in the same manner as that of Apatemon gracilis. In contrast with the latter, it rests for long intervals with the furcae spread wide apart, and the proximal fifth of the tail-stem and the body held at an angle to the rest of the tail-stem. This angle was most frequently acute, but could be as great as 120° on occasion (see Figure 15). No special structure could be seen in the tail-stem to account for its characteristic flexure. While resting, the preacetabular part of the body is bent slightly ventrally.

The dimensions of D. spathaceum cercariae from several sources are presented in Table 8. The general morphology of the cercaria is shown in Figure 12.

The mouth opens subterminally through the ventral side of the anterior organ. Two pairs of penetration gland ducts open antero-laterally to the mouth on each

side. The prepharynx appears to possess muscular walls, and the same comments apply as for Apatemon gracilis (page 48). A muscular pharynx lies a short distance behind the anterior organ. The long oesophagus runs posteriorly and slightly dorsally, bifurcating just before the ventral sucker into two broad and prominent caeca which terminate close to the level of the excretory bladder, and occupy most of the body dorsally.

Two pairs of large, granular, penetration gland cells with clear nuclei lie ventral to the caeca, and largely posterior to the ventral sucker. The ducts from the anterior pair of gland cells pass between the caeca to lie dorsal and lateral to the bases of these on either side. Here they meet with the ducts from the posterior gland cells, one on each side. These latter ducts lie just ventral to the caeca, passing round them laterally as the caeca turn inwards and ventrally towards the oesophagus. The two ducts on each side run forwards, lateral and slightly dorsal to the oesophagus, are thrown into a loop in the lateral plane at the level of the pharynx, and frequently undergo a dilation within the anterior organ.

The anatomy of the body is identical with that of the cercaria of Diplostomum sp. (Figures 13 and 14).

The tail-stem contains many small and irregular caudal bodies clustered along the excretory canal.

Spines forming the apical tuft anterior to the mouth are arranged in three transverse rows. The numbers of spines in 14 cercariae (derived from sticklebacks from the Forth and Clyde Canal) were, 16 spines (2 specimens), 17 spines (3), 18 spines (7), 19 spines (1), and 20 spines (1). The post-oral collar consists of approximately 40

spines in each of seven or eight regular and closely spaced transverse rows round the body. The anteriormost rows consist of large hooks, about 3μm long and directed backwards, giving way to much smaller spines posteriorly. In the ventral midline, several large hooks of the anteriormost row are absent, the posterior margin of the mouth occupying this region (Figure 21). Several very irregular and sparse rows of small spines occupy the region behind the post-oral collar almost back to the posterior edge of the anterior organ. Here lies the first of several rings of spines arranged regularily along the body to the level of the posterior edge of the ventral sucker (Figure 20, see also Figures 22 and 23). The first eight rings are complete, consisting of from 60 to 80 spines around the body. There are additional spines laterally associated with the first few rings, creating double rows for short lengths along each ring. The remaining three rings are complete dorsally, but are interrupted ventrally by the ventral sucker. The body posterior to the 10th ring has a sparse, irregular, covering of spines into which the 11th ring merges. These irregular spines are most abundant laterally and around the body at the level of the bladder. Spineless zones lie dorsally and ventrally behind the ventral sucker.

The ventral sucker (Figure 27) bears two complete concentric rings of spines, with a partially complete third ring anteriorly or laterally. This incomplete ring is of variable extent, being represented in some specimens by one or two spines, and in others by up to 20 spines. The spines of the outer complete ring are 2.5μm long. The number of spines in the outer complete ring of

17 cercariae (derived from metacercariae in the eyes of rainbow trout from College Mill Trout Farm) were as follows, 49 spines (3 specimens), 50 spines (0), 51 spines (5), 52 spines (5), 53 spines (3) and 54 spines (1). For four cercariae derived from metacercariae from sticklebacks (Forth and Clyde Canal), the corresponding numbers were 47, 48, 49, and 50 spines respectively.

The margins of the furcae bear small spines which were thought to continue along the tail-stem, although this could not be confirmed.

The distribution of sensory papillae is shown in Figures 29, 31, and 34. As in the cercariae of Apatemon spp., papillae are concentrated anteriorly and ventrally on the body. The pattern differs from that on either of the Apatemon species examined. On the body and furcae, considerable uniformity in arrangement of the papillae was observed. The distribution of those on the proximal part of the tail-stem is less consistent.

Nothing can be added to the description of the excretory system by Komiya (1938). The bladder arm runs forwards on each side to the level of the ventral sucker where it gives off a short, blind-ending, duct before curving back through several loops to its junction with the anterior and posterior collecting ducts. The recurved portion of the bladder arm contains three clusters of cilia. The anterior collecting duct on each side drains three flame cells from above the level of the pharynx to the ventral sucker. The posterior collecting duct drains two flame cells in the tail-stem, and collects tributaries from a further three in the posterior third of the body. The distributaries of the caudal excretory canal open midway along the dorsal margin of

each furca, immediately after a slight dilation of the duct. There is no excretory commissure. The flame cell formula is thus 2[(3)+((3)+(2))]= 16.

The caudal musculature of D. spathaceum cercariae appears identical to that of the cercaria of Diplostomum sp. described below, but was not studied in as great detail (see page 62).

Yellow patches were commonly seen anterior to the ventral sucker.

Diplostomum (D.) phoxini;

Cercariae of this species emerged from Lymnaea peregra collected at College Mill Trout Farm. Experimental completion of the life-cycle, starting from metacercariae in the brains of minnows (from Mugdock Reservoir, Milngavie, Dunbartonshire), yielded cercariae. Detailed morphological observations were only made on specimens from College Mill Trout Farm.

The dimensions (range and mean) of eight specimens fixed in hot formalin are as follows;

body length	99-116μm	(105μm)
maximum body width	33-51	(41)
tail-stem length	177-210	(200)
tail-stem width	27-43	(34)
furca length	156-180	(168)
anterior organ length	42-51	(46)
anterior organ width	26-36	(29)
ventral sucker length	19-24	(22)
ventral sucker width	20-34	(26)

Little can be added to the description of this cercaria by Rees (1957). It differs from the cercaria of

Diplostomum spathaceum in several respects. The cercaria of D. phoxini is substantially smaller than that of D. spathaceum (see discussion, page 77). It rests with the tail-stem straight and the preacetabular part of the body bent slightly ventrally (as does the cercaria of D. gasterostei, Figures 17 and 18). The tail-stem contains six pairs of large, regular, caudal bodies. Body spines are similar to those on D. spathaceum, but appear large relative to the smaller size of the body. There are nine rings of spines back to the level of the ventral sucker in the present form. Behind this are a further three irregular rows of small spines laterally, and posterior to these, a sparse scattering of small spines ventro-laterally to the junction of the body and tail-stem. Only the first few body rings are complete dorsally. The ventral sucker carries two rings of spines, with between 33 and 36 in each ring. It was not determined whether the tail-stem carried bands of spines.

The cercaria of D. phoxini cannot be separated from that of D. spathaceum on the basis of the excretory system, the relative length of the oesophagus, or the arrangement of sensory papillae. The anterior bladder arms of the present form possess a recurved portion containing three clusters of cilia. Rees (1957) stated that this feature was absent. The margins of the furcae bear small spines which were not mentioned by Rees. The arrangement of sensory cilia on the tail-stem of the present form agrees with that figured for D. spathaceum (Figure 34). Rees stated that her form possessed 12 hairs along each side of the tail-stem, regularily arranged along its length.

The description of the cercaria of D. phoxini by Arvy and Buttner (1954) is inadequate with respect to these comparative details.

Diplostomum (D.) gasterostei;

Cercariae of this species emerged from Lymnaea peregra collected at College Mill Trout Farm, from the Forth and Clyde Canal, and from flooded gravel pits at Packington, Warwickshire. Metacercariae from the eyes of perch (Loch Lomond), of 3-spined sticklebacks (Forth and Clyde Canal), and of rainbow trout (College Mill Trout Farm), were used in experimental completion of the life-cycle, in each case yielding cercariae (see also Table 3 and page 92).

Cercariae of D. gasterostei from all the above sources were examined in compiling the following description.

Dimensions of cercariae fixed in hot formalin are presented in Table 9.

As with D. spathaceum, the cercaria swims intermittently, hanging motionless in the water for long periods. At rest, the furcae are held apart, an obtuse angle between them. The tail-stem and body hang vertically downwards, with the preacetabular region of the body bent slightly ventrally (Figures 17 and 18).

The tail-stem contains six pairs of nucleated caudal bodies. These are not sufficiently large to completely fill the lumen of the tail-stem, are rather irregular in outline, and the distal three pairs are not entirely symmetrical about the excretory canal in ventral view.

The spination differs slightly from that of D. spathaceum. The apical tuft consists of between 6 and 11 spines (mode 9), arranged in three rows. For cercariae from various sources, the numbers counted are shown in Table 10.

There are 11 rings of spines on the body from the posterior of the anterior organ to midway along the ventral sucker, with additional spines laterally outwith the rings. Lateral to the ventral sucker, and extending to the posterior of the body, are irregularily arranged spines such as seen in D. spathaceum. The 9th-11th rings of spines merge with these laterally, the last two being vestigial. In cercariae derived from metacercariae in perch, the last two rings could not be distinguished from the irregular spines. Body rings on the majority of cercariae, regardless of source, were incomplete mid-ventrally from the 6th or 7th ring posteriorly, and ring 9 was the first interrupted by the ventral sucker. Mid-dorsally, the last few rings were frequently incomplete or very sparse. Specimens from the same source often showed variation in this respect.

The ventral sucker spination is very similar to that of D. spathaceum. Cercariae from the Forth and Clyde Canal and from College Mill Trout Farm have two complete rings of spines, a third incomplete ring of variable extent lying around the anterior part of the sucker. Three complete rings were observed on cercariae from Packington, and on those derived from metacercariae in perch (Figure 25). Cercariae from the latter source occasionally possessed an incomplete fourth ring anteriorly. Ventral sucker spines are about 3μm long. Table 11 gives the numbers of spines in the outermost complete ring of cercariae from various sources.

Four bands of spines were observed along the tail-stem, one either side of the mid-line, dorsally and ventrally. The bands continue onto the furcal margins where they are overlaid by the tegument, thus creating a narrow fin-fold (as in Figure 28). At the tips of the furcae, the fin-folds merge into a solid "nipple".

Specimens stained with silver nitrate revealed a distribution of papillae identical with that on D. spathaceum.

The only previous description of this cercaria is that of Williams (1966b). The present forms possess a recurved anterior bladder arm containing three clusters of cilia, and a fin-fold on the furcae, although neither feature was mentioned or figured by Williams. In addition, Williams states there to be "numerous small caudal bodies" within the tail-stem, in contrast with the six pairs in the present forms.

The cercaria of D. gasterostei cannot be distinguished from that of D. spathaceum by any feature not mentioned above.

Diplostomum (Diplostomum) sp.;

Cercariae of this species emerged from Lymnaea peregra collected at College Mill Trout Farm.

Between short bursts of swimming, the cercaria rests with the furcae wide apart, and the tail-stem flexed laterally a short distance along its length. The proximal fifth of the tail-stem and the body are thus held at an obtuse angle (between 135° and 180°) to the rest of the tail-stem (Figure 16).

The dimensions (range and mean) of 15 cercariae fixed and measured in hot formalin are as follows;

body length	211-243μm	(228μm)
maximum body width	51-61	(57)
tail-stem length	209-281	(253)
tail-stem width	38-48	(40)
furca length	209-272	(247)
anterior organ length	53-68	(61)
anterior organ width	25-30	(29)
ventral sucker length	25-34	(30)
ventral sucker width	27-34	(32).

This cercaria closely resembles that of D. spathaceum, although the tail-stem contains fewer caudal bodies. About 12 pairs, irregular and sometimes subdivided, are arranged along the excretory canal.

Slight differences in spination were also observed. The present form possesses an apical tuft of approximately 12 spines in 2-3 transverse rows. There are 11 rings of small spines arranged regularily to the posterior edge of the ventral sucker.(Figures 22 and 23). Rings 6-11 lack a few spines mid-dorsally, and rings 9-11 are interrupted by the ventral sucker. The ventral sucker bears two concentric rings of inwardly directed hooked spines (Figure 26). The larger spines of the outer ring are arranged alternately with the smaller spines of the inner. The outer ring contains 42-47 spines (counted in 11 specimens). In a small proportion of those examined, an incomplete third ring occurred anteriorly. Bands of spines on the tail-stem continue onto the margins of the furcae where a tegumentary membrane unites the spines into a conspicuous fin-fold (Figure 28).

The caudal musculature of all species examined was very similar, but was most easily observed in the cercaria of Diplostomum sp. Longitudinal bands of muscles lie along the tail-stem, one each side of the midline, dorsally and ventrally, and continue along the appropriate furca close under its dorsal or ventral margin. These muscles are attached under the furcal margin for most of its length, and onto the lateral (outer)face of each furca over its proximal half. Structures, probably representing longitudinal fibres, lie on the medial (inner) face of each furca, and entirely within the furca. All longitudinal fibres are striated in appearance. Superficial circular muscles give the tail-stem a ridged appearance, and dorso-ventral fibres under each face of the furcae are presumably analogous in function (see discussion, page 72). Other muscular elements such as described by Pearson (1961) for the cercaria of Neodiplostomum (=Fibricola) intermedium may occur, but were not observed.

The arrangement of sensory papillae, as revealed by silver nitrate staining, did not consistently differ from that of D. spathaceum.

Diplostomum (Tylodelphys)spp.;

On three occasions, cercariae referable to the subgenus Tylodelphys were obtained. These cercariae possessed two pairs of penetration glands in front of the ventral sucker, and an excretory system with the flame cell formula 2[(2+2)+((2)+(2))]= 16 and a preacetabular commissure. This agrees with the characters for cercariae of this subgenus in Dubois' key (Dubois, 1970a p 286).

On one occasion, cercariae emerged from a Lymnaea peregra collected in the Forth and Clyde Canal near Glasgow. A small proportion of perch in this canal were observed to contain Tylodelphys metacercariae in their eyes (see page 95).

Cercariae also emerged from one of a number of L. peregra exposed to fluke eggs from the faeces of a duckling fed eyes of rainbow trout from College Mill Trout Farm. The other snails in the experimental group shed the cercaria of Diplostomum gasterostei. No adult Tylodelphys were found on autopsy of the duckling some days after the collection of its faeces. Nor were metacercariae of this subgenus identified with certainty within rainbow trout from the fish farm. However, a single L. peregra from the fish farm was also found to be shedding Tylodelphys cercariae (see page 183).

No further work was done on these cercariae.

DISCUSSION

A) Comparison of the cercaria of Apatemon gracilis with related forms.

Four cercariae have been described which have the following in common with the cercaria of Apatemon gracilis; vestigial gut caeca, six pairs of large caudal bodies, a flame cell formula of 2[(2)+((2)+(1))]= 10, a postacetabular excretory commissure, and the habit of almost constant swimming once emerged from the snail host. These cercariae are, Cercaria hirsuta Miller, 1927; C. granula Miller, 1927; C. dohema Cort and Brackett, 1937;

and C. ancyli Johnston and Beckwith, 1947b.

Cercaria granula has about 26 nuclei in the postacetabular penetration gland mass, while C. hirsuta has about 12. C. dohema and C. ancyli possess three pairs of penetration glands, whereas the cercaria of A. gracilis described by Vojtek (1964) has four pairs, and the form described here, six pairs. As far as the published descriptions permit comparisons, the spination of all forms appears similar, and their dimensions do not greatly differ. Cercaria ancyli stands distinct by the possession of a spur on each furcal margin opposite the opening of the excretory canal, and of four pairs of "head glands" at the level of the anterior organ.

Crocombe (1959) described a cercaria, "Cercaria duodecaglandis", he considered to belong to A. gracilis, emerging from Ancylastrum fluviatile (Muller) in south Wales. This form is very similar to the present cercaria and possesses six pairs of penetration gland cells. Its excretory formula is $2[(2)+((3)+(1))]=12$, thus differing from the present form. It is an active swimmer and penetrated bullheads (Cottus gobio L.) to yield encysted metacercariae in the body cavity.

Two other species of cercariae also bear comparison with that of A. gracilis, although the description of neither is satisfactory. These are Cercaria F3 Petersen, 1931 and C. micromorpha Brown, 1926. The former is poorly figured and only briefly described by Petersen. His figure does show short, bulbous, caeca terminating well anterior to the ventral sucker, and a number of postacetabular penetration glands. C. micromorpha has the same excretory formula as the cercaria of A. gracilis,

but no transverse commissure was described. There are six pairs of caudal bodies and two pairs of post-acetabular penetration glands. A muscular pharynx is described, "but the oesophagus and intestinal caeca are apparently absent" (Brown, 1926).

The relationships to one another of cercariae in this group must await experimental completion of their life-cycles for clarification.

The arrangement of sensory papillae was worked out for cercariae of A. gracilis from a single source (see Figure 32). The only other Apatemon sp. for which this pattern was determined is A. (Australapatemon) minor (Figure 33). The two forms occupy different subgenera within Apatemon, and their papillary patterns are very different, as might be expected. It is noteworthy that the pattern observed on A. minor corresponds very closely with the arrangement figured by Richard (1971 plates 10 and 11) for her "Cercaria 1", suggesting that the latter may belong to the subgenus Australapatemon, and may represent A. minor. This is further evidence of the taxonomic value of sensory papillae in cercariae at the generic or subgeneric level.

B) Cercariae of the subgenus Diplostomum

1) Limits of the discussion

In his classification of cercariae of the subgenus Diplostomum, Dubois (1970a p278 et seq.) has included forms with 16, 18, and 20 flame cells, and with two or three pairs of penetration glands which may occupy several positions relative to the ventral sucker. All cercariae

described here within this subgenus possess 16 flame cells in their excretory systems, and two pairs of post-acetabular penetration glands. Dubois has based inclusion of other cercarial types on the two cases discussed below.

a) Despite differences in their excretory systems, Dubois (1966) has chosen to synonymise Cercaria scudderi Olivier, 1941 (20 flame cells) and the cercaria of Diplostomum baeri eucaliae Hoffman and Hundley, 1957 (16 flame cells) on the basis of dimensions, resting posture, number of caudal bodies, spination, and other features which, in the details discussed, are either common to many forms, or are unreliable (see discussion below). Dubois (1966) considered that Hoffman and Hundley (1957) may have been mistaken in their interpretation of the excretory system of D. baeri eucaliae. The only character in his (Dubois') comparison that appears almost unique to the two forms is the presence of three clusters of spines in the apical tuft (although this feature has also been described for Cercaria elodes Olivier, 1942) in contrast with the single cluster seen in other forms. This is not here considered sufficient, in the light of the described differences between their excretory systems, to justify synonymy of the two cercariae.

b) Cercaria micradena Cort and Brackett, 1938 possesses 20 flame cells, and two pairs of penetration glands, one pair pre- and the other postacetabular. Experimental completion of the life-cycle by Olivier (1940) yielded and adult worm which he assigned to the genus Diplostomum. Dubois (1953) considered this form a synonym of Hysteromorpha triloba (Rudolphi) until the demonstration of the life-cycle of the latter by Hugghins (1954a, b)

showed the synonymy to be invalid. Dubois (1970a p 320) later placed Diplostomum micradenum in the subgenus Diplostomum.

Unambiguous experimental evidence is therefore lacking to justify inclusion of Cercaria scudderi and C. micradena in the subgenus Diplostomum. The discussion here of cercariae in this subgenus will be limited to forms with 16 or 18 flame cells and two pairs of postacetabular penetration glands.

2) Inter- and intra-specific variations of Diplostomum cercariae found during this study.

This section of the discussion is based primarily on the cercariae of D. spathaceum and D. gasterostei. Comparisons of these from several sources during this study and from the literature indicate limits of inter- and intra-specific morphological variations.

For D. spathaceum, several descriptions of the cercaria with experimental study of the life-cycle to confirm identity, are available in the literature. These include accounts by Cort and Brooks, 1928 (Cercaria flexicauda, Michigan, U.S.A.); Wesenberg-Lund, 1934 (Denmark); Johnston and Clelland, 1938 (Cercaria murrayense, Australia); Komiya, 1938 (Germany); Cichowlas, 1961 (brackish water of the southern Baltic); Shigin, 1968a (cercaria of D. indistinctum, U.S.S.R.); and Sweeting, 1971 (England). Some morphological features of these forms are included in Table 12. All these forms have in common with the cercaria of D. spathaceum described here, a characteristic flexure of the tail-stem while resting, numerous caudal bodies,

and all undergo further development in the lens of fish eyes.

The only previous record of the cercaria of D. gasterostei has been mentioned earlier (page 60).

The dimensions of the above populations of D. spathaceum cercariae, together with those of populations described here, are presented in Table 8. The variation is considerable. For example, the body length of the cercaria of the Australian form (as stated by Johnston and Clelland, 1938) is 138-185μm, and that of the Russian form (Shigin, 1968a) is 205-250μm. However, these apparently discontinuous samples are overlapped by several intermediate in size. As indicated in Table 8, uniform fixation methods are not always used by authors. Although it is easy to ascribe differences between samples to differing fixation methods, it is noteworthy that there is no overlap in tail-stem length between rainbow trout-derived cercariae and cercariae from Packington, despite the fact that both samples were prepared and measured in an identical manner.

Of the dimensions of D. gasterostei from various sources, only tail-stem length is consistently different from the dimensions recorded for D. spathaceum (Tables 8 and 9). Even here, there is considerable overlap which would render difficult separation of the species on this basis.

The literature suggests the spination of cercariae has some value in systematics. Unfortunately, the majority of authors have paid insufficient attention to the numerical data which may be obtained, for example, by counting spines in the apical tuft and on the ventral sucker.

Of the descriptions of the cercaria of D. spathaceum listed above, three give definite statements of the numbers of spines in the apical tuft. These are, "15 spines in two rows" (Komiya, 1938), 11 spines (Shigin, 1968a), and 10-13 spines (Sweeting, 1971 p 102) (see Table 12). In the present study, cercariae derived from metacercariae in sticklebacks from the Forth and Clyde Canal possessed 16-20 spines in the apical tuft. Thus a range of between 10 and 20 spines in the apical tuft is established for the cercaria of D. spathaceum.

The cercariae of D. gasterostei examined here possessed between 6 and 11 spines (mode 9) in their apical tuft, only 7 out of 56 examined possessing 10 or 11 spines. A more or less clear distinction between the cercariae of D. spathaceum and D. gasterostei may be drawn on this basis.

On the ventral sucker of cercariae of D. gasterostei occur two or three complete rings of spines, and an additional incomplete ring. That of D. spathaceum carries two complete rings with a third ring incomplete. The spines are similar in shape in both species, but the numbers in each differ. In the outer complete ventral sucker ring of D. spathaceum cercariae, between 47 and 54 spines were counted. From the literature, counts of "120 in two rows" (Komiya, 1938), 49-58 in each ring (Shigin, 1968a), and 48 spines (Sweeting, 1971), give limits of 47 to about 60 spines in each complete ventral sucker ring of D. spathaceum. The number of spines counted in 27 specimens of D. gasterostei cercariae lay between 35 and 46 in the outer complete ventral sucker ring. There was thus no overlap with the number observed on D. spathaceum cercariae.

In this study, it was not considered feasible to separate D. spathaceum and D. gasterostei cercariae on the basis of the number and arrangement of rings of spines on the body behind the post-oral collar. In both species, 11 rings are present and the number and extent of additional spines forming double rows is variable. The last two or three rings (at the level of the ventral sucker) were vestigial in most cases, consisting of a few spines laterally, and merging with the irregularily arranged lateral spines posterior to this. Indeed, in D. gasterostei cercariae derived from metacercariae in the eyes of perch, the last two rings of body spines were not apparent.

The majority of descriptions in the literature agree in general with the above arrangement of body rings. These include descriptions by Johnston and Clelland (1938) and Shigin (1968a) for D. spathaceum, and Williams (1966b) for D. gasterostei. Cort and Brooks, (1928), however, described a rather different spination pattern for Cercaria flexicauda. In this form, they reported 18 rings of body spines from behind the post-oral collar to the posterior end of the body. Cichowlas (1961) reported for the cercaria of D. spathaceum from the Baltic, that "the entire body is covered with rows of very small spines". Whether these two observations are accurate or are mis-interpretations of the cercarial structures is impossible to say. No such pattern was observed during this study. Cercaria maritzburgensis Porter, 1938 and C. nassa Martin, 1945, as described by Stunkard, 1973, (in addition to C. laruei and C. modicella both also described by Cort and Brooks, 1928) are other strigeoid cercariae with rings of spines to the posterior of the body.

Bands of fine spines on the tail-stem continuous with those on the margins of the furcae were observed on all cercariae examined for this feature. Previous records of caudal spine bands are few. Pearson (1956, 1959, 1961) has described such bands on cercariae of Alaria spp., Strigea elegans, and Neodiplostomum intermedium. Probert (1966a) figured similar bands on the tail-stem of the cercaria of Apatemon minor. Such spine bands are extremely difficult to observe. As they have been described from widely differing genera in the Strigeidae and the Diplostomatidae, they may prove to be general rather than exceptional among strigeoid cercariae.

Marginal spines on the furcae of D. gasterostei cercariae are covered by a tegumentary fringe to produce a narrow fin-fold. This differs from the broader fin-folds of schistosome cercariae in which supporting spines are absent. Fin-folds were also observed in cercariae of Diplostomum sp., and have been reported by Shigin (1968a, 1969a) for the cercariae of D. indistinctum (=D. spathaceum) and of D. gobiorum. The latter closely resembles the cercaria of D. gasterostei. It is of interest that fin-folds were not observed in any samples of D. spathaceum cercariae from Britain. British specimens bear very small spines on the furcal margins. The width of a fin-fold depends on the length of spines within the furcal tegument, a character which could possibly be subject to intra-specific variation.

Silver nitrate staining of the sensory papillae of D. spathaceum and D. gasterostei cercariae revealed no consistent difference between these (Figures 29, 31, and 34). Identical patterns were also observed on cercariae of D. phoxini and Diplostomum sp., and corresponded

exactly with the pattern figured by Richard (1971) for D. spathaceum from France. Although similar patterns were figured by Shigin (1968a, 1969a) for cercariae of D. indistinctum and D. gobiorum, Richard suspected that Shigin had ommitted a few papillae from his diagrams. The striking agreement between Richard's figure for D. spathaceum, and the four species of Diplostomum examined here, is taken as confirmation that the subgenus is very homogeneous with respect to the distribution of sensory endings.

The numbers of "tail-stem hairs", long cilia arising from papillae on the tail-stem, could only be determined accurately in specimens stained with silver nitrate. Several authors (see Table 12) have figured or counted these hairs on Diplostomum cercariae. No importance is placed on the numbers of these unless silver nitrate staining was used in their study.

Studies of aspects of behaviour may reveal species-specific patterns. Sweeting (1971 p 106) reported that cercariae of Diplostomum petromyzi-fluviatilis will rest for several minutes between bursts of swimming, suspended from the surface film, in contrast to the more active pelagic behaviour of D. spathaceum. It is regretted that more behavioural studies were not undertaken.

The swimming action of all strigeoid cercariae appears very similar. It has been described earlier (page 47) for Apatemon gracilis and by Graefe and Burkert (1972) for Diplostomum spathaceum. The caudal musculature in each case consists of four bands of longitudinal muscles running along the tail-stem and into the furcae. These are responsible for the lateral flexing of the tail during swimming. Turgor is maintained

within the continuous cavities of the tail-stem and furcae by these, and by the circular muscles of the tail-stem, and the dorso-ventral fibres of the furcae. No qualitative differences between the musculature of Apatemon gracilis cercariae and that of the Diplostomum spp. studied could be detected.

The excretory system has been subject to more misinterpretation in the literature than perhaps any other anatomical feature of the cercaria. Where descriptions of Diplostomum cercariae are brief, it is often difficult to decide whether reliance should be placed on an author's interpretation of, for example, the nature of excretory commissures, or the the clusters of cilia in the recurved bladder arm. In all cercariae of the subgenus Diplostomum examined, three clusters of cilia are present in the bladder arm. However, Cort and Brooks (1928) figure Cercaria flexicauda as lacking both the recurved portion of the bladder arm and its clusters of cilia, as does Williams (1966b) for D. gasterostei. Several authors (Cichowlas, 1961; Wesenberg-Lund, 1934; Sweeting, 1971) make no comment about this part of the excretory system in D. spathaceum. Wesenberg-Lund (1934) described the cercaria of D. spathaceum as possessing a preacetabular commissure, a feature never seen during the present study, nor otherwise mentioned in the literature.

Cichowlas (1961) gives the flame-cell formula of D. spathaceum cercariae from the Baltic as $2[(3)+((5)+(1))]= 18$. This is one more flame cell posteriorly in the body and one fewer in the tail-stem than in Cercaria X. Each therefore has a total of 18 flame cells, as opposed to 16 for D. spathaceum cercariae

Taken at face value, the results of Cichowlas indicate that Cercaria X might not be distinct from the cercaria of D. spathaceum. It should be pointed out that, although Cichowlas experimentally obtained metacercariae within the lens of fish eyes, she did not perform feeding experiments to obtain adult worms for identification. In addition, it is entirely possible that she "mis-read" the excretory formula of her cercaria.

The gut caeca of all Diplostomum cercariae examined were septate and wrinkled in appearance to a greater or lesser extent. The point of bifurcation of the oesophagus is always slightly anterior to the ventral sucker. No importance is attached to gut topography within the species examined.

Faint, irregular, yellow patches were frequently noted slightly anterior to the ventral sucker of cercariae of D. spathaceum and of Diplostomum sp. A similar feature is reported by Johnston and Clelland (1938) for Cercaria murrayense (= D. spathaceum). These probably represent the "refractile yellow globules" as described for Cercaria X by Erasmus (1958), and which have been used in species separation by various authors. Erasmus (1958) suggested that such yellow patches might represent glycogen deposits. Their presence within the above Diplostomum spp. suggests they are of little taxonomic value.

Some conclusions as to specific criteria in Diplostomum cercariae will be summarised now. Dimensions appear of little value in this respect. Intra-specific variations, which may be exaggerated by differing fixation methods, mask any specific differences. The resting posture of cercariae and the number of their caudal bodies

show remarkable constancy within a species. These characters are easily observed, and are taken here as a primary means of separating species or species groups. The value of spination remains uncertain largely because of inadequate descriptions in the literature. From this study, it seems that that the numbers of spines in the apical tuft and on the ventral sucker are diagnostic aids. The arrangement of spines elsewhere on the body was similar in all species studied. The few differing spine patterns described in the literature may be due to misinterpretation or may reflect variants not seen here. The presence or absence of a fin-fold is a possible, if dubious, systematic character. Other features discussed above appear of little or no significance within the range of material studied.

3) Extension of the discussion to other cercariae of the subgenus Diplostomum.

The described Diplostomum cercariae with 16 or 18 flame cells and two pairs of postacetabular penetration glands fall conveniently into three groups on the basis of resting posture and caudal body numbers.

a) Within the first group are all those forms with the tail-stem bent through about 90°, and containing many small caudal bodies. This includes all cercariae referable to D. spathaceum discussed above. In addition, Cercaria paracauda Iles, 1959, C. helvetica XIII Dubois, 1929, Furcocercaria I Odening, 1962, and Cercaria X clearly belong within this group (Table 12).

D. spathaceum is the only adult species shown experimentally to have a cercaria in this group. Indeed, it is possible that all the above cercariae can be

referred to this species. Dubois (1970a p 348) considers C. helvetica XIII and Furcocercaria I Odening synonyms of D. spathaceum on morphological grounds. He also considers (Dubois, 1970a p 280) C. paracauda a synonym of Cercaria X despite the differences in their flame cell formulae. C. paracauda cannot be separated from the cercaria of D. spathaceum on the morphological criteria examined in the previous section of this discussion and summarised on page 74. C. paracauda, Cercaria X, and the cercaria of D. spathaceum all develop further in the lens of fish eyes.

A number of other cercariae deserve mention as possible members of this group. Although the resting posture of Cercaria helvetica XV Dubois, 1929 is unknown, the number of its caudal bodies and the presence of a furcal fin-fold suggest affinities with the cercaria of D. indistinctum (=D. spathaceum). The description of Cercaria chromatophora by Brown (1931) gives neither its resting posture nor the number of caudal bodies. Wikgren (1956), considering its yellow patches to be diagnostic of this species, applied the name C. chromatophora to cercariae probably belonging to D. spathaceum. Erasmus (1958) considered C. chromatophora a possible synonym of Cercaria X. Furcocercaria Nr 2 Petersen, 1931 has been synonymised with D. spathaceum by Dubois (1970a p 348). However, the original description of the former (see also Table 12) neglected the majority of taxonomically important features.

b) Within the second group, the cercariae rest with their tail-stems straight. In all cases a small number, generally six pairs, of large caudal bodies occupy much of the tail-stem lumen (see Table 13). Included here are

the cercariae of Diplostomum phoxini, D. gasterostei, D. baeri eucaliae, D. gobiorum Shigin, 1965, and D. petromyzi-fluviatilis (Diesing, 1850). The adults of all five species are small worms with the genitalia anteriorly placed in the hindbody. The first three species are considered particularily closely related (discussion in Hoffman and Hundley, 1957 and Dubois, 1970a p 310), thus similarities in their cercariae are not unexpected. Adult forms are not known for the remaining cercariae in this group. These are Cercaria laruei Cort and Brooks, 1928; C. yogena Cort and Brackett, 1937; C. maritzburgensis Porter, 1938; and Cercaria fennica IV Wikgren, 1956 (see also Table 13).

Where known, the next host for cercariae of this group is a fish or cyclostome. Cercariae of Diplostomum phoxini, D. baeri eucaliae, and D. petromyzi-fluviatilis localise in the brains of minnows (Phoxinus phoxinus), brook sticklebacks (Eucalia inconstans), and lampreys (Lampetra spp.) respectively. D. gasterostei cercariae localise in the retina or humours of various fish (see page 92), and Cercaria laruei and that of D. gobiorum in lenses. The record of C. maritzburgensis penetrating the eyes of goldfish (Porter, 1938 p 412) is suspect.

All the above forms possess six pairs of caudal bodies except Cercaria fennica IV (5-7 pairs) and the cercaria of D. petromyzi-fluviatilis (five pairs).

There is a wider variation in cercarial dimensions than in the group containing D. spathaceum. D. gobiorum (body length 122μm) and D. phoxini (125μm) may clearly be separated from D. gasterostei (about 200μm), D. petromyzi-fluviatilis (242μm), and D. baeri eucaliae

(203μm). Cercaria laruei (body length 169μm), C. yogena (172μm) and C. maritzburgensis (145-200μm) are intermediates.

The apical tuft of spines, where accurately counted, is generally sparser than in the first group of cercariae. Cercariae of D. phoxini possess five, of D. gasterostei 6-11 (mode 9), of D. gobiorum seven, and of D. petromyzi-fluviatilis 6-10. Even a slight variation on the quoted figures would rule out separation of species on the basis of this character except for the cercaria of D. baeri eucaliae. The possession of three clusters of spines in the apical tuft of the latter is a feature otherwise only seen in Cercaria scudderi Olivier, 1941 and C. elodes Olivier, 1942 (see also page 66).

The spination of the ventral sucker has been described in detail for only a few forms. The cercaria of D. gasterostei has two or three complete rings each consisting of 35-46 spines. Those of D. phoxini and D. gobiorum bear two rings, each containing respectively 36 spines and "about 31" (Shigin, 1969a). The cercaria of D. petromyzi-fluviatilis has a row of long hairs. Only the last named species appears to be clearly separable on the basis of its ventral sucker spination.

The cercaria of D. phoxini possesses 8-9 rings of spines, of which most are incomplete dorsally, between the post-oral collar and the ventral sucker. Both Cercaria laruei and C. maritzburgensis have rings back to the junction of the body and tail-stem (see also page 70). The remaining forms possess 9-12 rings back to the level of the ventral sucker.

The excretory system, where fully described, is identical to that observed for the cercariae of D. spathaceum and D. gasterostei. Exceptions are the cercaria of D. baeri eucaliae in which Hoffman and Hundley (1957) observed cilia in the caudal excretory duct at the top of the tail-stem, Cercaria laruei and C. maritzburgensis for which no recurved bladder arms with ciliary clusters were reported, and the cercaria of D. petromyzi-fluviatilis for which Sweeting (1971) gave the flame cell formula as 2[(2)+((4)+(2))]= 16. The same comments apply as on page 73.

Cercaria yogena is the only form in this group reported to contain yellow patches.

A further two species which might belong to this group are Cercaria chrysenterica Miller, 1923 and C. elodes Olivier, 1942 (see Table 13). Both rest with their tail-stems straight, have two pairs of postacetabular penetration glands, and a small number of caudal bodies. Although both have 18 flame cells, by analogy with the case of Cercaria X, they might be referable to the subgenus Diplostomum. C. chrysenterica penetrates fish (Cort and Brooks, 1928) and C. elodes, tadpoles. The dimensions of the two forms are within the range of those for D. gasterostei, a species which they resemble in most respects. However, C. elodes is distinguishable by its apical tuft arranged in three groups. The constriction behind the ventral sucker in resting cercariae of this species, on which stress was placed by Olivier (1942), may be seen in other forms as a consequence of the ventral bending of the body in this region. C. elodes also possesses yellow patches in front of the ventral sucker.

Cercaria modicella Cort and Brooks, 1928 is a small form (body length 123μm) for which the excretory system was not fully worked out. It differs from C. laruei in only a few details, and may be referable to this group of cercariae.

Comparisons of forms within this group of cercariae lead to conclusions similar to those for the first group. For all forms with similar resting postures and numbers of caudal bodies, the characters which might be of taxonomic use are often ambiguous and difficult to determine accurately. No characters for absolute separation of species are apparent, although it is generally possible to separate species for which good descriptions exist. Thus the cercaria of D. phoxini and of D. gobiorum may be distinguished from virtually all other forms on the basis of their dimensions, a character otherwise of little value. Likewise, the cercaria of D. petromyzi-fluviatilis has five pairs of caudal bodies, in contrast with the six pairs of most other species in the group, and a single row of hairs on the ventral sucker.

c) The cercaria of Diplostomum sp. does not conveniently fit into either of the above groups. It possesses about 12 spines in its apical tuft, two rings of about 45 spines on the ventral sucker, 11 rings of spines on the body back to the level of the ventral sucker, fin-folds on the furcae, and yellow patches in front of the ventral sucker. In its excretory system, as in the above details, it scarcely differs from either D. spathaceum or D. gasterostei. However, its caudal body number and resting posture clearly distinguish it from any described form.

SECTION 2 THE METACERCARIAE

INTRODUCTION

Strigeoid cercariae, after penetrating a fish host, migrate through its tissues to their favoured sites. Here they grow and develop over a period of days or weeks into metacercariae. It was hoped that studies on fish host specificity of cercariae and their subsequent localisation and development within the fish would augment morphological data as a means of distinguishing between species.

MATERIALS AND METHODS

A) Sources of fish

Fish used for experimental infections were obtained from the following sources; 3-spined sticklebacks (Gasterosteus aculeatus L.) from Lochan na Lairige near Loch Tay, Perthshire; 9-spined sticklebacks (Pungitius pungitius (L.)) from the Forth and Clyde Canal, Glasgow, and from a pond in Dawsholm Park, Glasgow; minnows (Phoxinus phoxinus (L.)) and stone loach (Noemacheilus barbatulatus (L.)) from the River Almond at College Mill Trout Farm; brown and rainbow trout fingerlings (Salmo trutta L. and S. gairdneri Richardson) from Howietoun and Northern Fisheries, Bannockburn, Stirling; young perch (Perca fluviatilis L.) from Loch Lomond, and goldfish (Carassius auratus (L.)) from commercial suppliers in Glasgow. Sticklebacks from Lochan na Lairige and trout from Howietoun were free of strigeoid infection when obtained. Fish from all other

sources contained small numbers of metacercariae. After fish had been kept in the laboratory for some weeks, newly penetrated cercariae from experimental infections could be readily distinguished from earlier infections.

B) Experimental infection of fish

The sources of cercariae of each species used are indicated where appropriate in the text.

For experimental infections, suspensions of recently emerged cercariae were added to containers each holding a group of one species of fish. The concentration of cercariae in the initial suspension was estimated by subsampling 1ml aliquots. Care was taken to ensure that the cercarial suspension and the water in the containers were brought to the same temperature (approximately 15°C).

To establish numbers and location of newly penetrated cercariae, fish were generally killed between 24 and 48 hours after exposure to infection. By this time, most cercariae could be expected to have localised in their favoured site (Erasmus, 1959; Ratanarat-Brockelman, 1974). After experiments with Diplostomum cercariae, the eyes and brains of the fish were removed to separate dishes of teleost saline and teased apart. Cercariae were counted and picked out with a pipette over a period of at least an hour. The body and pericardial cavities of fish exposed to Apatemon gracilis were washed out, and the viscera examined, in addition to the eyes and brain. The diverse localities in which newly penetrated Apatemon cercariae were found made accurate counts difficult. For this reason, some fish were kept for several weeks after infection before

examination for metacercariae.

C) Treatment of metacercariae

Dimensions of free-living or excysted metacercariae were taken from specimens fixed without compression in hot formol saline, stained with Gower's carmine, and mounted in balsam.

Encysted metacercariae could be freed by subjecting them to pepsin and trypsin-tauroglycocholate solutions. Cysts were placed for 10 minutes at 40°C in a 0.8% solution of pepsin in Hanks' saline adjusted to pH 1.7-2.0. They were then washed in several changes of saline, and incubated at 40°C in a solution of 0.5% trypsin and 0.3% sodium tauroglycocholate in saline, adjusted to pH 7.0. This is a standard technique used in the Wellcome Laboratories for Experimental Parasitology of Glasgow University for excystation of cestode cysticercoids (Hopkins, personal communications).

Histochemical tests on metacercariae were based on Humason (1972, pp 281-2 and 308-9). For demonstration of calcareous bodies, live metacercariae were placed in a 0.5% solution of alizarine red S buffered to pH 9.0 (phosphate buffer). Calcareous bodies, and to a lesser extent, the ducts of the reserve excretory system, stain orange-red within a few minutes. Frozen sections of organs containing metacercariae were stained with Sudan black B to demonstrate lipids. Haematoxylin and eosin were used routinely to stain wax sections examined for location and pathology of worms.

RESULTS

Apatemon (A.) gracilis;

A) Infection of fish

1) Experimental infections

Cercariae of A. gracilis from Iceland (see Chapter 5) penetrated and developed in 3-spined sticklebacks, but not in 9-spined sticklebacks.

In another series of experiments carried out under as nearly uniform conditions as possible, the following fish were exposed to A. gracilis cercariae emerging from Lymnaea peregra collected at College Mill Trout Farm; 28, 3-spined sticklebacks; six, 9-spined sticklebacks; 25 rainbow trout; 9 brown trout; four goldfish; three perch; and two stone loach. The fish were autopsied at intervals after exposure to infection. All brown and rainbow trout became infected. Those 3-spined sticklebacks exposed to large numbers of cercariae (several hundred/fish) were found to contain cercarial bodies if autopsied the next day. However, metacercariae were never found subsequently. In one case, the body cavity of a stickleback contained a single dead cercarial body four weeks after exposure to infection. None of the 9-spined sticklebacks, perch, goldfish, or stone loach became infected.

There appeared to be no significant difference between brown and rainbow trout with respect to numbers of cercariae penetrating, and subsequent localisation of metacercariae within the body. The total numbers of metacercariae recovered from various parts of the bodies of four trout, 14 weeks after their experimental

infection were; pericardial cavity, 1706 (87%); body cavity (most clustered on the pyloric caeca), 203 (10%); cranial cavity and orbit, 38 (2%); and eye, 17 (1.0%). Metacercariae from experimentally infected rainbow trout yielded adult A. gracilis when fed to ducklings (see Table 1).

2) Natural infections

Table 14 presents data on the distribution of Apatemon spp. metacercariae in naturally infected fish from several localities. The location of metacercariae within the fish varies according to the fish species. In trout, the great majority occur in the pericardium with smaller numbers elsewhere. In sticklebacks the majority occur in the eye, and in stone loach, in the body cavity.

Feeding experiments with metacercariae from trout, 3-spined sticklebacks, and stone loach from College Mill Trout Farm, and with 3-spined sticklebacks from Iceland, yielded adult A. gracilis (Table 1). Feeding experiments with metacercariae in perch from Loch Lomond failed to yield adult worms.

B) The metacercaria

1) Structure and development

The development of cercariae into metacercariae was not studied in detail. One trout, autopsied four weeks after infection and maintained at 15°C, contained 68 large unencysted metacercariae and 39 encysted forms. The unencysted forms (Figures 35 and 36) were presumed to be about to form a cyst. They were broad and flat, with a poorly differentiated hindbody. The holdfast was

represented by two lips, the posterior lip crescentic in ventral view with the oval anterior lip lying in the concavity of the crescent. Both lips were small, and scarcely overlapped the ventral sucker. The deep concavity of the fully developed metacercaria had not yet developed, but there was a slight hollow ventrally in which lay the ventral sucker and holdfast. The lappets were poorly developed, although the proteolytic gland at the base of the holdfast was apparent. No structures were visible which might be responsible for cyst formation. The range of dimensions of five specimens fixed in hot formol saline was;

body length	655-1020μm
body width	416-530μm
oral sucker length	49-72μm
oral sucker width	53-61μm
ventral sucker length	57-72μm
ventral sucker width	61-72μm
holdfast length	125-148μm
holdfast width	122-160μm

No metacercariae were seen that were clearly undergoing cyst formation.

Parasite cysts from all sources were cylindrical to egg shaped, and frequently surrounded with a capsule of host origin (Figures 43 and 44). The translucent wall of the parasite cyst is quite uniform in thickness, and lamellar in appearance. The opaque metacercaria is tightly confined by the cyst wall. The dimensions of 10 cysts from the pericardium of a rainbow trout were 542-660μm (mean 605μm) by 356-426μm (mean 395μm). The inner cavity containing the metacercaria was 465-542μm (mean 503μm) by 279-310μm (293μm).

2) Excystation of the metacercaria

The process of excystation was observed on several occasions. In pepsin, the outer host capsule is gradually digested, and may be freed from the parasite cyst by agitation. The outer lamellae of the cyst wall begin to slough off, separating most from the cyst at the pole where the worm will emerge. Initial movement of the parasite within the cyst ceases after a few minutes, and does not occur again until after excystation. Excystation was never seen to occur without trypsin treatment. In trypsin, the wall at one pole becomes very thin as successive lamellae peel back, and a bulge may appear at this point (Figure 39). Excystation is a very rapid, explosive incident. The metacercaria is ejected along with a mass of lipid droplets, and comes to lie 1-2mm away from the cyst while the lipid droplets rise slowly to the surface of the medium. Usually the worm emerges with the anterior end first. Sometimes part of the worm remains within the cyst, from which it never completely frees itself. Normal excystations are accompanied by a contraction of the cyst wall, layers of which are thus thrown into diagonal criss-cross folds (Figures 40-41). The central cavity of the cyst is much smaller after expulsion, and the wall itself is thicker.

This excystation sequence was also seen in a high proportion (over 50%) of cysts from a rainbow trout deep frozen for five months. In a few cases, complete rupture of one end of the cyst was not acheived, a small hole appearing terminally through which the contents were extruded in a narrow stream.

The excysted metacercaria resembles the forebody of the adult worm with a rudimentary hindbody attached

(Figures 37 and 38). The deep forebody concavity, opening antero-ventrally, is fully formed and contains the large holdfast and ventral sucker. Distinct lappets are present either side of the oral sucker. The rudiments of the genitalia in the hindbody stain with Gower's carmine. The distinct proteolytic gland lies at the base of the holdfast, marking the boundary between the forebody and the future hindbody. The large interconnected lacunae of the reserve excretory system are packed with refractile droplets. The Sudan black B test revealed these to be lipid in nature (Figure 47). No calcareous material could be demonstrated.

Excysted metacercariae remain active in warm (40°C) saline. At intervals they expel quantities of lipid droplets through the terminal excretory pore. The droplets then float up to the surface. The dimensions of excysted metacercariae from trout and loach are presented in Table 15.

C) Pathology

As mentioned earlier, metacercariae are frequently enclosed by a fibrous host capsule. This is most marked in the pericardium of heavily infected trout, where cysts appear to lie embedded within a mat of fibrous tissue (Figure 52). Dead, unencysted, metacercariae were often seen encapsulated within the pericardium. An encapsulation response by the host is evident against metacercariae in other sites (Figures 42, 43 and 44).

Apatemon (A.) annuligerum (Nordmann, 1832) Odening, 1970;

Odening (1970), in Germany, obtained adults of this

species in a buzzard (Buteo buteo) fed cysts from perch eyes. Dubois (1974) considers this species separate from A. gracilis.

Metacercariae obtained from the eyes of Loch Lomond perch during this study (Table 14) failed to yield adult worms when fed to ducklings and black-headed gulls (Table 1). Dubois (1974 in litt.) commented that metacercariae from these perch, which were sent to him, have in common with the metacercaria of A. annuligerum, a ventral sucker much larger than the oral sucker. Metacercariae from the eyes of Loch Lomond perch are therefore tentatively assigned to A. annuligerum.

Cotylurus (Ichthyocotylurus) variegatus;

Metacercariae of this species were found in perch from Loch Lomond. Details are given earlier, on page 17.

Diplostomum (D.) spathaceum;

A) Infection of fish

One series of infection experiments was carried out using cercariae emerging from a single large Lymnaea peregra from Packington. Table 16 summarises the data obtained. Only a small proportion of cercariae established themselves in perch and in goldfish. In minnows, rainbow trout and sticklebacks, however, a much larger proportion reached the lens where they established themselves under the lens capsule proximally. No cercariae were seen to localise elsewhere in the eye.

The following species of fish were found to be naturally infected with lens metacercariae presumed

to be referable to either D. spathaceum or Diplostomum sp., 3-spined and 9-spined sticklebacks, roach (Rutilus rutilus (L.)), perch, pike (Esox lucius L.), and common eel (Anguilla anguilla (L.)) from the Forth and Clyde Canal; brown and rainbow trout, stone loach, and 3-spined sticklebacks from College Mill Trout Farm, and perch from Loch Lomond.

B) The metacercaria

The metacercaria has been described several times from European fish (von Nordmann, 1832; Komiya, 1938; Sweeting, 1971 p57). Its brief description here will also suffice for other metacercariae known to belong to this subgenus, since these resemble one another closely (Figure 45). The body is spatulate and flattened with a subterminal posterior bud, arising slightly dorsally, representing the future hindbody. The oral sucker is flanked by a pair of lappets. In the posterior half of the body is the oval holdfast, slit longitudinally, with the ventral sucker lying immediately anterior to it. An extension of the excretory ducts of the cercaria has produced a branching primary excretory system comprising many flame cells. The ducts of the reserve excretory system connect many small vacuoles each containing a round calcareous body.

Twenty metacercariae from rainbow trout (experimentally infected with cercariae derived from roach metacercariae) were measured.

body length	334-465μm	(mean 396μm)
body width	133-186μm	(157μm)
oral sucker length	32-42μm	(37μm)
oral sucker width	36-42μm	(39μm)
ventral sucker length	30-38μm	(34μm)

ventral sucker width 30-40μm (mean 35μm)
ratio; (distance from posterior edge ventral sucker to posterior edge body)/body length
= 0.29-0.39 (mean 0.35).

C) Pathology

Little can be added to published accounts of the pathology in fish of D. spathaceum infections (see Ferguson and Hayford, 1941; Sudarikov, 1960a; Ashton et al., 1969; and Sweeting, 1971 pp 115-129). Exophthalmia, herniations of lens material on cercarial entry, liquefaction of the lens cortex, invasive proliferation of the lens epithelium leading to a cataractous condition, and overgrowth of the pupil, were regularily observed in both naturally and experimentally infected trout (Figures 48 and 49).

Diplostomum (D.) phoxini;

Metacercariae of this species were found in the brains of minnows from Mugdock Reservoir, Milngavie, from Loch Lomond, and from College Mill Trout Farm. Twenty specimens were fixed in hot formol saline and measured;

body length	258-341μm	(mean 302μm)
body width	137-159μm	(146μm)
oral sucker length	40-49μm	(45μm)
oral sucker width	36-51μm	(40μm)
pharynx length	21-36μm	(25μm)
pharynx width	15-27μm	(21μm)
ventral sucker length	36-48μm	(39μm)
ventral sucker width	38-46μm	(42μm)
holdfast length	49-61μm	(56μm)
holdfast width	55-68μm	(63μm)

ratio; (distance from posterior edge ventral sucker to posterior edge body)/body length =0.39-0.46 (mean 0.42)

Diplostomum (D.) gasterostei;

A) Infection of fish

Infection experiments were carried out using D. gasterostei cercariae from naturally infected Lymnaea peregra from the Forth and Clyde Canal, Glasgow; naturally infected L. peregra from Packington; and experimentally infected L. peregra (exposed to miracidia derived from metacercariae in perch from Loch Lomond). The results of these experiments are shown in Tables 17, 18, and 19.

All cercariae localised in the retina, except in the case of trout, where the majority appeared to be within the vitreous humour. Sticklebacks were the most susceptible to infection by D. gasterostei cercariae from all sources. It is of note that perch could only be infected experimentally by cercariae derived from metacercariae in perch (Table 19). Cyprinids used in these experiments were largely refractory to infection.

Metacercariae thought to represent D. gasterostei were found in the eyes of 3-spined and 9-spined sticklebacks from the Forth and Clyde Canal, of brown and rainbow trout from College Mill Trout Farm, of 3-spined sticklebacks and perch from Loch Lomond, and of bullheads (Cottus gobio L.) from Black Loch, Fenwick Moor, south of Glasgow. In trout, metacercariae localised in the vitreous humour, and in sticklebacks and perch, they were in the retina. Table 3 summarises feeding experiments, where carried out, to verify these

identifications.

B) The Metacercaria

Twelve metacercariae from experimentally infected sticklebacks were measured;

body length	353-418μm	(mean 384μm)
body width	190-224μm	(208μm)
oral sucker length	42-51μm	(48μm)
oral sucker width	36-49μm	(45μm)
pharynx length	27-36μm	(30μm)
pharynx width	21-29μm	(28μm)
ventral sucker length	42-48μm	(45μm)
ventral sucker width	48-53μm	(50μm)
holdfast length	76-101μm	(86μm)
holdfast width	82-95μm	(89μm)

ratio; (distance posterior edge ventral sucker to posterior edge body)/body length

= 0.43-0.48 (mean 0.45)

C) Pathology

There is no gross pathology associated with infections of this metacercaria. Infected fish are normal in appearance, respond to shadows falling over them, and are capable of feeding on food presented. Sections of infected stickleback eyes show metacercariae in the pigment layer proximal to the layers of rods and cones. There may be considerable local disruption of the pigment layer, and some damage to the layers distal to this (Figures 50 and 51). No inflammatory responses were observed. The tendency of metacercariae to localise peripherally in the eye, rather than close to the exit of the optic nerve, may minimise impairment of vision. In rainbow trout, where metacercariae localise in the

vitreous humour, no pathological changes could be ascribed to these alone, since concurrent infections of Diplostomum spp. were present in all fish examined.

Diplostomum (Diplostomum) sp.

A) Infection of fish

Cercariae of this species were only obtained from Lymnaea peregra collected at College Mill Trout Farm. Preliminary experiments indicated that the cercaria penetrates 3-spined and 9-spined sticklebacks, goldfish, minnows, and brown and rainbow trout. The proportion reaching the eye and localising in the lens was very low except in both trout species. No significant difference was observed between numbers localising in brown and in rainbow trout. The cercaria appeared not to penetrate perch or stone loach. The results of one series of quantitative experiments are shown in Table 20.

B) The metacercaria

The dimensions of 20 metacercariae of Diplostomum sp. from the lens of a rainbow trout 12 weeks after its infection, are as follows;

body length	467-574μm	(mean 522μm)
body width	201-247μm	(228)
oral sucker length	46-53μm	(50μm)
oral sucker width	44-53μm	(47μm)
pharynx length	29-36μm	(30μm)
pharynx width	21-27μm	(24μm)
ventral sucker length	38-44μm	(41μm)
ventral sucker width	42-48μm	(45μm)
holdfast length	87-107μm	(100μm)
holdfast width	72-93μm	(81μm)

ratio; (distance posterior edge ventral sucker to posterior edge body)/body length

= 0.37-0.41 (mean 0.39)

C) Pathology

As with D. spathaceum, cercariae penetrate the lens over its proximal half, and most often close to the pupil. Cercariae then migrate within the cortex of the lens to lie close together proximally. See comments on D. spathaceum (page 91) for observations on pathology and natural occurrence.

Diplostomum (Tylodelphys) spp.

Within the humours of a small proportion of perch from the Forth and Clyde Canal were found metacercariae of two species within the subgenus Tylodelphys. Such metacercariae are more elongated than those in Diplostomum, and possess poorly developed lappets, an indistinct hindbody, and oval calcareous bodies.

One of these forms was very large, no individuals being under 1mm long. By its size and structure, it would seem to belong to Diplostomum (Tylodelphys) podicipinum Kozicka and Niewiadomska, 1960, occurring in perch, burbot (Lota lota(L.)), and ruffe (Gymnocephalus cernua (L.)) in Poland (Kozicka and Niewiadomska, 1960). Similar metacercariae have been reported from perch in England (Sweeting, 1971 p77), and (as Diplostomulum scheuringi) from yellow perch (Perca flavescens (Mitchill)) in North America (Hughes, 1929b).

The smaller metacercaria in perch from the Forth and Clyde Canal was presumed fully developed, since it contained many calcareous bodies in an extensive reserve

excretory system. It may be referable to Diplostomum (Tylodelphys) clavatum von Nordmann, 1832, a common parasite of perch (Dubois, 1970a p 371).

No experimental work was done on either form.

DISCUSSION

A) Metacercaria of Apatemon gracilis and related forms

There are several descriptions in the literature of fully developed metacercariae belonging to the subgenus Apatemon. That of A. gracilis has been described by Yamaguti (1933)(= A. pellucidus), Hoffman (1959), Crocombe (1959), and Vojtek (1964)(=A. cobitidis). The metacercaria of A. annuligerum has been described by Odening (1970) and Kozicka (1961,1972) and of a further Apatemon sp. by Kozicka (1972). Yamaguti (1933) described the metacercaria of A. fuligulae, a small form occupying a thin-walled cyst. With the exception of A. fuligulae, all the above metacercariae are very similar in their morphology. Dubois (in litt.) has commented on the much larger size of the ventral sucker than of the oral sucker in the metacercaria of A. annuligerum (see also Odening, 1970, and Kozicka, 1972). The suckers are more equal in size in A. gracilis.
A detailed morphological study of all the above forms is not considered worthwhile here. Details of their development, dimensions of cysts, and aspects of their biology are discussed below.

1) Development of the metacercaria and structure of the cyst

Little new information has been added here to the few previous descriptions of metacercarial development. However, it is possible to draw parallels between the process in Apatemon gracilis and that in other members of the Strigeidae. Szidat (1929) and Wesenberg-Lund (1934 p 146) followed the development of Cotylurus cornutus metacercariae in snails. Once within a snail, the cercaria grows to become a large flattened form, granular in appearance, in which cercarial features are lost or diminished. A decrease in size follows, during which metacercarial features appear, and the body darkens just prior to cyst formation. Similarily, metacercariae of A. gracilis presumed about to encyst, are flattened, larger than the encysted form, and with a poorly developed holdfast and hindbody. Kozicka (1961) reported similar large, pre-encystment, metacercariae for A. annuligerum, as did Crocombe (1959) for A. gracilis. Vojtek (1964) described the decrease in size of A. cobitidis (=A. gracilis) metacercariae prior to cyst formation, and the way in which the cyst itself decreases in size as its walls become thicker. In this way, the metacercaria comes to be tightly compressed within the cyst, a phenomenon also observed in Cotylurus cornutus.

All cysts of Apatemon spp. recovered during this study were similar in size and shape to those described by Yamaguti (1933) for A.pellucidus (=A. gracilis) from the body cavity of Mogurnda obscura in Japan, and by Kozicka (1961) for A. annuligerum in perch eyes. The cyst poles are occasionally extended to give a lemon-shaped

cyst in A. cobitidis (=A. gracilis)(Vojtek, 1964), a variant not noted here. Cysts from the musculature of the brook stickleback (Eucalia inconstans) in North America are rather larger than those in the present study, and have a projection of the host capsule at one end (Hoffman, 1959). All authors have commented on the difficulty of mechanical dissection of the above cysts. (The cysts of Apatemon fuligulae are much smaller than those of A. gracilis, and the worm may be freed without difficulty (Yamaguti, 1933)).

The lamellar nature of some trematode cyst walls has been observed by Erasmus and Bennett (1965) for Holostephanus luhei Szidat, 1936 and Cyathocotyle bushiensis Khan, 1962, both in the Cyathocotyloidea (see Appendix 1). Erasmus (1967a) considers the similar lamellae in cyst walls of Fasciola hepatica (see Dixon and Mercer, 1964) to differ in structure and function from those of the former two species.

2) Excystation

It has been observed that, during excystation, a metacercaria may aid rupture of its cyst by movement and probing (Erasmus and Bennett, 1965; Dixon, 1966). It was suggested by Dixon (1966) that secretions by the metacercaria of Fasciola hepatica may weaken part of the cyst wall. Neither phenomenon need occur in the excystation of Apatemon gracilis since metacercariae killed by freezing are ejected from cysts in the normal manner when treated with pepsin and trypsin.

The contents of A. gracilis cysts appear to be under pressure, causing an explosive expulsion of the enclosed worm, accompanied by a decrease in the volume

of the cavity and a slight thickening of the contracted cyst wall. There appears to be no previous record of such a mode of excystation in trematodes. The principle behind the expulsion of worms from A. gracilis cysts remains obscure. The criss-cross diagonal ridges in the wall of the empty cyst may indicate the position of elastic fibres, or may simply be warping following contraction. It is difficult to imagine such an expulsion of the worm from thin-walled cysts in which the metacercaria occupies only part of the central cavity (e.g. in Posthodiplostomum spp.). The excystation of Cotylurus cornutus, in which the cyst wall tightly invests the metacercaria, was observed during this study. Once in warm pepsin, the worm becomes very active, distorting the cyst wall which appears to adhere to its tegument. Excretory bodies are extruded through a posterior pore in the cyst. Excystation appears to result from a gradual digestion of the cyst wall (in which no lamellations are visible), until the tegument of the worm is exposed to the medium.

Excretory bodies in Diplostomum metacercariae are demonstrably calcareous, and those in Apatemon gracilis, lipid (Figures 46 and 47). Cable (1965) suggested that formation of calcareous bodies provides a means of immobilising excretory materials in encysted metacercariae which might be unable to expel these. Lipid excretion has been demonstrated in several adult strigeoids, the metacercariae of which possess calcareous bodies. The changeover in excretory products begins in the fully developed metacercaria or young adult (Erasmus, 1967b; 1972 p 223). Calcareous bodies could not be demonstrated in Apatemon gracilis either

before cyst formation or in fully developed metacercariae. Thus any change in excretory metabolism between the metacercarial and young adult stages is not apparent in this species.

B) The metacercariae of Diplostomum spp.

The remarkable uniformity of metacercariae within the subgenus Diplostomum makes their identification difficult. Sweeting (1971 ppl-43), in reviewing the larval genus Diplostomulum, has considered this problem, and no attempt at a complete survey of the group will be made here.

In view of the developmental changes undergone by these metacercariae, and the problems of uniform preparation, measurements are of little value in species separation. Williams (1966b) considered the position of the ventral sucker of value in differentiating between two forms (D. spathaceum and D. gasterostei) in the eyes of sticklebacks. This position may be expressed as a ratio, (distance from the posterior edge ventral sucker to posterior edge body)/body length. This ratio for the present metacercariae is;

D. spathaceum	0.29-0.39 (mean 0.35)
D. phoxini	0.39-0.46 (0.42)
D. gasterostei	0.43-0.48 (0.45)
Diplostomum sp.	0.37-0.41 (0.39).

In the above forms, position of the ventral sucker, and location within the host proved sufficient for recognition, although this proved difficult between D. spathaceum and Diplostomum sp. However, these

characters cannot be used as a sole means of separating all described diplostomula (Sweeting, 1971 p 78).

Shigin (1965a, 1968b) has placed importance on the numbers of calcareous bodies in the reserve excretory system. He proposed several metacercariae as new species on this basis, without taking into account the influence of host species or age of infection, and without experimental continuation of the life-cycle. Difficulty was found in determining the numbers of calcareous bodies during this study. In addition, only very few were seen in some metacercariae of D. spathaceum, even many weeks after infection of the fish.

Authors have used the location of a metacercaria as a clue to its identity. The site occupied within a fish by metacercariae of any Diplostomum sp. is remarkably constant (eye or brain). Diplostomum spathaceum only occurs in the lens (although D. huronense, an American form synonymised with the former, is recorded from the vitreous and lens of percids (Hughes and Hall, 1929)). Unfortunately, location is not sufficient on its own. Of species for which adults are known, Diplostomum sp. (this study), D. gobiorum (see Shigin, 1969a), D. mergi (see Shigin, 1965b), D. rutili Razmashkin, 1969 (see Razmashkin, 1969), and D. commutatum (Diesing, 1850)(see Shigin, 1969b) also occur within the lens. Similarily, D. gasterostei and D. pusillum (Dubois, 1932)(see Agapova and Galieva, 1972) both occur in the retina, and D. phoxini, D. baeri eucaliae (see Hoffman and Hundley, 1957), and D. petromyzi-fluviatilis (see Sweeting, 1971 p 69) are in the brains of fish or lampreys.

The host within which a metacercaria is found is

likely to be a useful aid to its identification only where a species is known to be restricted to certain hosts. D. phoxini may come into this category, being known only from minnows (genus Phoxinus). However, the list of natural and experimental fish hosts of many strigeoids (e.g. D. spathaceum and Apatemon gracilis) is constantly being extended. The existence of different races in several species may further confuse the issue (see page 107).

C) Host responses to the metacercaria

It is of interest that Apatemon cysts in the brain ventricles and vitreous humours of fish eyes become invested with a host capsule (see also Kozicka, 1958), whereas Diplostomum phoxini metacercariae in the brain ventricles, and other Diplostomum spp. in the eye, are not so affected. Similar host encapsulation (granuloma formation) around schistosome eggs in mammals is a cell-mediated immune response (Warren, 1972). Both eye and brain are considered "immunologically privileged sites". The full implications of this concept are not yet understood (Billingham and Silvers, 1971 pp 64-69). Both sites wholly or partially lack lymphatic drainage, and it appears that, in mammals at least, antigenic material transplanted into the brain ventricles or anterior chamber of the eye is most liable to be destroyed if the recipient has been previously sensitised (Medawar, 1948). Not all cercariae penetrating a fish can be expected to localise successfully. A number die en route, and are destroyed by the host (Erasmus, 1959) thus presumably supplying an antigenic stimulus. Moreover, metacercariae of Apatemon gracilis do not

encyst, even at summer temperatures (see page 85), for at least 4 weeks, and those of Posthodiplostomum minimum for 19-26 days (Hoffman, 1958). Crocombe (1959) found some unencysted Apatemon gracilis in the body cavity of bullheads 74 days after their experimental infection. In all cases, a parasite cyst was formed in advance of the host capsule. At the temperatures used for infection and maintenance of rainbow trout in this study, a humoral immune response may be mounted by the fish within 4 weeks (Ridgeway et al., 1966). It is possible that an Apatemon metacercaria may be able to withstand the immune response for a time, perhaps by coating itself with host antigen as has been demonstrated for schistosomes (as reviewed by Clegg, 1972). However, its survival may ultimately depend on formation of a protective cyst. It is significant that host capsules develop around encysted metacercariae which remain viable, whereas any encapsulated but unencysted metacercariae appear dead. Chubb (1964) observed that plerocercoids of the psuedophyllidean cestode Triaenophorus nodulosus in perch remained alive, perhaps for many months, encapsulated in the liver, but eventually became degenerate and were destroyed.

The only fish-infecting strigeoid metacercariae which never form a cyst are those such as Diplostomum, occurring exclusively in the eye or brain. It has commonly been assumed that localisation in these "immunologically privileged sites" has freed the worm from the necessity of forming a protective cyst. However, the host response to cysts of Apatemon gracilis in these sites (Figure 44) indicates that some other explanation might be necessary. It may be that, by

living in the eye or brain, a metacercaria will not continue to stimulate the immune system as it may elsewhere in the body, and thus will avoid the full immune onslaught. Nevertheless, since the fish is capable of responding against cysts in these sites, some form of antigenic camouflage by the metacercaria may still be necessary.

D) Pathology of strigeoid infections in fish

Metacercariae of Apatemon spp. have not been implicated in mortality of fish. Heavy infections of other encysted strigeoid metacercariae have been found to cause loss of weight (Hunter and Hunter, 1938, discussing Crassiphialia (=Uvulifer) amploplitis) and other pathological effects leading to death (also reviewed by Kozicka, 1958 for Posthodiplostomum minimum). No severe damage could be demonstrated in any of the regions of the body where Apatemon gracilis was found. However, Kozicka (1958) observed various changes in the eyes of perch infected with Tetracotyle sp. 2 (= Apatemon annuligerum), and in the brains of young cyprinids infected with another Apatemon metacercaria (Tetracotyle sp. 1). Cysts in both these sites became invested with a capsule of fibrous host tissue.

The presence of Diplostomum metacercariae in the lens may cause visual impairment or blindness in two ways. Large numbers of worms may cause liquefaction of the lens cortex, presumably by their movement or by secreted substances, thus altering the optical properties of the lens. Very commonly, the epithelium of an infected lens may proliferate to produce an opaque cataract. Sallman

et al. (1966) have demonstrated that it is possible to induce such a growth in rainbow trout fed on carcinogens. Ashton et al. (1969) have commented on the similarities between the tumour-like growth reported by Sallman et al. and the cataract appearing on the lens of trout infected with metacercariae of Diplostomum spp. In the case of trematode cataract, it cannot be certain whether the invasive proliferation of the lens epithelium is initiated by chemical alteration of the underlying lens, or a response to physical injury of the epithelium. Rafferty (1963) has demonstrated that the epithelium of a frog lens will proliferate locally around a wound, giving a tumour-like growth. Rafferty commented on the differing susceptibilities of amphibian lenses to such proliferation after injury. It may be that fish also differ in their susceptibilities. The impression was gained during this study that rainbow trout lenses would become cataractous at infection levels more tolerated by species such as roach. That cataracts may be induced by a variety of factors is suggested by the observation that they may be associated (along with other degenerative changes) with wrong nutrition in rainbow trout (Hess, 1937).

Other pathological changes in the eyes of fish infected with Diplostomum spathaceum have been discussed further by Ashton et al. (1969) and by Sweeting (1971 pp115-129).

Infection of a fish eye with D. gasterostei metacercariae in the retina does not yield as dramatic a pathology as do lens metacercariae. Distortion and destruction of the retina by cysts of Posthodiplostomum spp. located there has been reported by Kozicka (1958).

E) Host and location specificity of metacercariae

Perhaps the most valuable data obtained from infection experiments is that concerning host and location specificity of cercariae and young metacercariae.

It may be true to say that all families of freshwater fish are parasitised by Diplostomum spp. the range of hosts parasitised by any one species may be narrow or wide. D. phoxini is known only from minnows of the genus Phoxinus. By contrast, few species appear exempt from experimental infection with D. spathaceum cercariae (see lists in Sudarikov, 1960a) which are also infective experimentally to several amphibians, reptiles, and mammals (Ferguson, 1943a). Posthodiplostomum minimum and Bolbophorus confusus (Krause, 1914) are other strigeoids with a considerable range of intermediate hosts (Hoffman, 1958; Olson, 1966).

Despite its wide occurrence, the susceptibilities of different fish species to infection with D. spathaceum vary. In the present study, minnows and rainbow trout appeared the most susceptible. Betterton (1973) demonstrated that rainbow trout are more susceptible than brown, and Sweeting (1971 Table 15) compiled a table showing minnows the most readily infected. A similar phenomenon was noted for D. gasterostei and Diplostomum sp. In the case of D. gasterostei, sticklebacks appeared the most liable to infection by cercariae from all sources. However, only cercariae experimentally derived from metacercariae in perch were capable of infecting perch. Cyprinids did not become infected, although Sweeting (1971 p 66) recorded this metacercaria from roach, chub, and bream,

as well as sticklebacks, perch, and grayling from England. Similarily, Apatemon gracilis cercariae from snails collected in the field could not be made to infect several fish species naturally infected with the metacercaria.

Such results suggest that more than one variety or race may occur in some species. The inclusion of more than one race in Posthodiplostomum minimum has been recognised for many years (Ferguson, 1943b). Hoffman (1958) proposed two subspecies, P. m. minimum of which cercariae are only infective to cyprinids, and P. m. centrarchi, infective to centrarchids. Observations by Bedinger and Meade (1967) that cercariae of P. minimum are of more than one morphological type strengthens the idea of race differences in this species. In the present forms, cercarial structure appears very constant within a species.

The use of "subspecies" as an intraspecific taxon implies a degree of genetic isolation between populations. It is preferred here not to use this taxon. Cercariae of D. gasterostei from all sources, for example, experimentally infected sticklebacks regardless of their differing specificities towards other fish. Metacercariae from all sources can mature in the same definitive hosts. The term "race", although vague, is preferred to "subspecies".

Caution is necessary in interpreting data about host specificity and susceptibility from naturally infected fish. Fish occupying different regions of a body of water will differ in their exposure to cercariae and therefore in their parasite burdens. This has been demonstrated by Wierzbicki (1971) and Styczynska-Jurewicz

(1959).

Other causes of fish host specificity are poorly understood. It should be borne in mind that, since the association of two animals is involved, features of either or both may be responsible for dictating specificity. As discussed above, cercariae of different races may differ innately in their abilities to infect any given host species. The reasons why cercariae from a single source should differ in their ability to establish in separate fish species may be more complex. That cercariae may enter a fish, but then fail to establish, has been indicated several times. Betterton (1973) has demonstrated that similar numbers of cercariae of D. spathaceum penetrate both brown and rainbow trout. Once within the former species, however, a far larger proportion fail to localise than in rainbow trout. Newly penetrated Apatemon gracilis could be found in the body cavity of 3-spined sticklebacks the day after exposure to large numbers of cercariae from College Mill Trout Farm (see page 84). Subsequently, no developing metacercariae were found in any tissue. Crocombe (1959) found the cercariae of A. gracilis from Wales would penetrate guppies (Lebistes reticulatus (Peters)) and bullheads in infection experiments, but would only develop further in the latter. Avoult and Smitherman (1965) observed that 12 fish species, when exposed to cercariae of Posthodiplostomum minimum, exhibited signs of irritation, suggesting cercarial penetration, although only a few species became infected. It remains a matter for conjecture whether host specificity arising after penetration of the cercaria is due to an immune response by the host, or to

physiological inadequacies of the cercaria. Whichever may be the case, cercariae, once dead, are soon destroyed by the host (Erasmus, 1959).

It may be significant that Apatemon gracilis metacercariae in naturally infected 3-spined sticklebacks are largely confined to the eyes (Table 14), although cercariae are also found in the body cavity immediately after experimental exposure. It is possible that cercariae reaching the eyes are sufficiently isolated from the immune response (see also page 103) to develop normally, whereas those in the body cavity are destroyed.

No acquired immunity to strigeoid metacercariae has been demonstrated in fish (Bauer, 1959 p 159; Donges 1964). With increasing size of fish, however, cercariae may find it increasingly difficult to reach their favoured site. Ratanarat-Brockelman (1974) reported that cercariae of D. spathaceum died if they had not reached the lens after migrating for some 12cms within a fish. Such a size-resistance phenomenon may partially explain observations by Kozicka (1958) that infection rates of several strigeoids decrease with age in fish. In the majority of cases, however, numbers of metacercariae are directly proportional to the age of their host (Sweeting, 1971 p 62; Spall and Summerfelt, 1969; Bibby, 1972).

Several aspects of the location specificity of metacercariae within their host have been discussed above. The remarkable degree of location specificity of Diplostomum metacercariae has been commented upon (page 101). Ratanarat-Brockelman (1974) reported that newly penetrated D. spathaceum cercariae dying before they reach the lens are encapsulated, even within the

retina, a site favoured by D. gasterostei. In concurrent infections of D. spathaceum, D. gasterostei, and Diplostomum sp., in trout eyes, there appear to be no competitive interactions leading to each species occupying a narrower habitat than in a single species infection.

SUMMARY

1) The cercaria of Apatemon gracilis was described and figured. Comparisons with related cercariae were hindered by ignorance of the specific identity of these.

2) Cercariae of four species within the subgenus Diplostomum were described. These were D. spathaceum, D. phoxini, D. gasterostei, and Diplostomum sp. Their cercariae could be separated on the basis of resting posture and numbers of caudal bodies, with details of spination a secondary character. The value of these and other specific criteria which have been used in cercarial taxonomy was discussed.

3) The development and structure of the metacercaria of Apatemon gracilis were briefly described. The process of excystation of the fully developed metacercaria was compared with that in related species. Experimental fish host and location specificities of the cercaria and metacercaria were compared with the natural occurrence of the metacercaria.

4) Experimental host and location specificities of the above Diplostomum spp. metacercariae were listed, along with a brief description of each. Pathology, and host and location specificity of strigeoids in fish were discussed.

TABLE 8: Cercaria of *D. spathaceum*: Dimensions

		Derived from metacercariae in rainbow trout	From Packington, Warwickshire	According to Komiya, 1938	According to Dubois, 1929	According to Cort & Brooks, 1928
Body	Length	153 - 207 (188)	173 - 209 (196)	173 - 197 (188)	180 - 200	139 - 231 (170)
	Width	33 - 48 (42)	42 - 51 (48)	49 - 71 (55)	45 - 55	43 - 62 (54)
Tail Stem	Length	234 - 264 (252)	209 - 230 (222)	191 - 229 (211)	180 - 225	216 - 293 (254)
	Width	30 - 45 (37)	30 - 40 (34)	30 - 37 (32)	27 - 34	31 - 54 (36)
Furca	Length	222 - 247 (228)	206 - 232 (222)	184 - 229 (215)	180 - 250	216 - 231
Anterior Organ	Length	51 - 63 (58)	55 - 65 (59)	49 - 56 (52)	40	50
	Diam.	24 - 33 (27)	25 - 29 (27)	30 - 35 (33)	25	-
Ventral Sucker	Length	21 - 30 (28)	25 - 27 (26)	30 - 33 (32)	25 - 31	35
	Width	27 - 33 (29)	29 - 30 (30)	30 - 33 (32)	25 - 31	35
No. Specimens		(10)	(10)	-	-	-
Method of Fixation		Hot formalin measured in the fixative	Hot formalin measured in the fixative	Heat killed	-	Killed in hot formalin

(range and mean, in µm) of specimens from various sources

According to Shigin, 1968a	According to Sweeting, 1971	According to Johnston & Clelland, 1938	According to Wesenberg-Lund 1934	According to Cichowlas, 1961
205 - 250 (228)	226	138 - 185 (158)	173 - 197	165 - 270
65 - 86 (75)	59	32 - 46 (37)	60	60
160 - 190 (175)	178	161 - 208 (188)	180	180
-	37	24 - 34 (27.5)	23 - 47	21
-	195	154 - 200 (173)	195 - 212	195
68 - 75 (71)	44	45 - 60	35 - 45	54
28 - 30 (29)	25	-	23 - 29	39
45 - 60 (51)	31	22 - 29	22 - 39	-
28 - 30 (29)	33	22 - 27	22 - 39	-
(20)	-	-	-	-
Mounted and measured in balsam	Freshly killed cercariae in 10% buffered formalin	-	"preserved"	-

TABLE 9: Dimensions (range and mean, in μm) of Diplostomum gasterostei cercariae. Measured in ventral view.

		According to Williams, 1966b	From Forth and Clyde Canal	Experimentally derived from metacercariae in L. Lomond perch	From College Mill Trout Farm	From Packington, Warwickshire
Body	Length	230 - 290 (250)	186 - 246 (215)	174 - 216 (193)	204 - 249 (227)	190 - 247 (219)
	Width	40 - 60 (50)	51 - 63 (55)	39 - 48 (45)	45 - 57 (52)	48 - 59 (53)
Tail-stem	Length	320 - 480 (330)	243 - 276 (261)	288 - 324 (308)	258 - 282 (270)	281 - 300 (295)
	Width	40 - 50 (40)	36 - 45 (41)	36 - 42 (39)	36 - 45 (41)	38 - 42 (40)
Furca	Length	270 - 330 (270)	234 - 270 (251)	225 - 261 (253)	261 - 275 (269)	258 - 285 (270)
Anterior Organ	Length	-	51 - 63 (57)	48 - 54 (51)	60 - 66 (62)	55 - 68 (63)
	Width	-	30 - 33 (32)	27 - 33 (29)	27 - 33 (30)	29 - 38 (32)
Ventral Sucker	Length	30 - 30 (30)	27 - 30 (28)	21 - 24 (23)	24 - 30 (27)	29 - 30 (30)
	Width	30 - 30 (30)	27 - 33 (30)	24 - 27 (25)	30 - 36 (31)	29 - 32 (30)
No. Specimens		(10)	(12)	(12)	(10)	(15)

TABLE 10; Numbers of spines in the apical tuft of Diplostomum gasterostei cercariae from various sources

Source of Cercariae	No. of Spines 5	6	7	8	9	10	11	Totals	No. of specimens
Forth and Clyde Canal	1	7	9	4	4			25	
Gravel Pits at Packington		2	3	5	2			12	
College Mill Trout Farm					2	2	2	6	
Exptlly. from metacercariae in L. Lomond perch		1	0	1	8	2	1	13	

TABLE 11: Spine counts from the outer ventral sucker ring of D. gasterostei cercariae

No. of specimens	Source of cercariae	No. of spines in ring												
		35	36	37	38	39	40	41	42	43	44	45	46	Total
	Forth & Clyde Canal	1	1		1				1					4
	College Mill Trout Farm							1	3	5	2	1	2	14
	Experimentally derived from metacercariae in perch		3	1	2	2	1							9

TABLE 12: Some characteristics of cercariae of Diplostomum

Cercaria and Reference	Resting posture and localisation in fish	Yellow patches in body	Arrangement of tail-stem hairs	Numbers of caudal bodies
1. D. spathaceum present form (see text)	Lens	Present	4 pairs distally, several (Fig. 34) proximally	Very numerous
(= Cercaria flexicauda) Cort & Brooks, 1928	Lens	-	"Hairs extend from tail stem on each side"	"Over 25"
(= Cercaria C.) Wesenberg-Lund 1934	Lens	-	-	Numerous
(= Cercaria C.) Komiya, 1938	Lens	-	Irregular on either side of tail stem	Numerous
(= Cercaria murrayense) Johnston & Clelland, 1938	Lens	Present	-	About 40
Cichowlas, 1961	Lens	-	Present	Numerous
(= D. indistinctum) Shigin, 1968a	Lens	-	Similar to present form	11 pairs, may be subdivided on emergence
Sweeting, 1971	Lens	-	7 pairs on tail-stem	42 caudal bodies
Richard, 1971	-	-	As in present form	-
2. Closely related forms Cercaria helvetica XIII Dubois, 1929		-	-	Numerous
Furcocercaria Nr. 2 Petersen, 1931	-	-	-	-
Cercaria chromatophora Brown, 1931	-	Present	7 hairs on each side	-
Cercaria X Erasmus, 1958	Lens	Present	14 hairs irregularly arranged	25 - 30 pairs
Cercaria paracauda Iles, 1959	Lens	Present	6 pairs of hairs	Numerous
Furcocercaria I Odening, 1962		-	10 pairs of hairs on each side	-

of Diplostomum spathaceum from different sources, and of related forms

Numbers of caudal bodies	Excretory formula etc.	Arrangement of apical tuft spines	Numbers of rings of spines on body	Arrangement of ventral sucker spines	Arrangement of spines on furcae
Very numerous	2[(3) + ((3) + (2))] = 16 (see text)	16 - 20 in 3 rows	11 to level of ventral sucker	2 complete rings of about 50 spines + 1 incomplete ring	Small spines on margins
"Over 25"	2[(3) + ((3) + (2))] = 16 bladder arm not recurved or ciliated	"About 10"	18 rows back to posterior of body	"About 2 rings of spines"	Scattered spines on furcae
Numerous	Pre-acetabular commissure present description poor	-	-	2 rings of spines	"No fin-folds"
Numerous	As in present form	15 in 2 rows	-	2 rows totalling 120 spines	Scattered spines on furcae
About 40	As in present form	About 12 spines	9 double rings to level of ventral sucker	2 rings	-
Numerous	2[(3) + ((5) + (1))] = 18	-	"Entire body covered with rows of very small spines"	2 rings	-
11 pairs, may be subdivided on emergence	As in present form	11 spines	9 rings; first few double	2 rings 49 to 58 in each	Fin-fold present, apparently lacking spines
42 caudal bodies	2[(2) + ((4) + (2))] = 16	10 - 13 spines	-	2 rings of 48 spines	-
-	-	-	-	-	-
Numerous	2[(3) + ((3) + (2))] = 16 bladder arm not recurved or ciliated	10 spines in 2 rows	"Several rings"	2 rings	-
-	-	3 alternating rows	Extend in double rows to level of pharynx	-	-
-	2[(3) + ((4) + (2))] = 18 with recurved, ciliated bladder arm	-	Present	2 - 3 rings	-
25 - 30 pairs	2[(3) + ((4) + (2))] = 18 bladder arm not recurved or ciliated	18 spines	8 rings back to ventral sucker	2 rings	Spines on furcal margins
Numerous	As in present form but only two ciliary clusters in bladder arm	17 spines in 3 rows	10 rings back to ventral sucker	3 rings	Spines on furcal margins
-	As in present form	Present	11 - 12 rings back to ventral sucker	2 rings	Furcae finely spined

TABLE 13: Some characters of Diplostomum cercariae and of related forms, which rest with their tail stems straight.

Cercaria and Reference	Localisation in host	Yellow patches in body	Numbers of caudal bodies	Excretory formula etc.	Arrangement of apical tuft spines	Numbers of rings of spines on body	Arrangement of ventral sucker spines	Mean body length	Mean tail stem length
Cercaria of D. gasterostei (present forms)	Retina or humcurs of fish	-	6 large pairs	2[(3) + ((3) + (2))] = 16	6 - 11 spines (Mode 9)	9 - 11 rings to level of ventral sucker	35-46 in each of 2 or 3 complete rings	>200 µm (fixed)	about 270 µm
Cercaria of D. phoxini Rees, 1957	Brain of minnow	Absent	6 large pairs	As D. gasterostei but bladder arm not recurved or ciliated	5 spines	8 rings to level of ventral sucker most incomplete dorsally	double ring 36 in each	125 µm (fixed)	200 µm
Cercaria of D. baeri eucaliae Hoffman & Hundley, 1957	Brain of brook stickleback	Absent	6 large pairs	16 flame cells not fully determined	3 groups of spines	9 - 11 evenly spaced rings to ventral sucker	3 irregular rings	200 µm (fixed in hot formalin)	232 µm
Cercaria of D. gobiorum Shigin, 1969a	Lens of fish	-	6 large pairs	As D. gasterostei	7 in 2 rows	12 rings last 6 incomplete	2 rings about 31 spines in each	122 µm	130 µm
Cercaria of D. petromyzi-fluviatilis Sweeting, 1971	Brain of lampreys	-	5 large pairs	2[(2) + ((4) + (2))] = 16	6 - 10 spines	-	single ring of long hairs	242 µm	275 µm
Cercaria chrysenterica Miller, 1923 & Cort & Brooks, 1928	Penetrate fish localise in eye	-	4 - 8 or more pairs	2[(3) + ((4) + (2))] = 18	Present	9 rings to ventral sucker	-	260 µm	244 µm
Cercaria laruei Cort & Brooks, 1928	Lens of fish	-	6 large pairs	As D. gasterostei but bladder arm not recurved or ciliated	About 15 spines	About 17 rings to posterior of body	About 4 rings	169 µm (fixed in hot formalin)	283 µm
Cercaria modicella Cort & Brooks, 1928	-	-	6 large pairs	not fully worked out	About 10 spines	About 17 rings to posterior of body	2 rings	123 µm (fixed in hot formalin)	185 µm
Cercaria yogena Cort & Brackett, 1937	-	Present	6 large pairs	As D. gasterostei	About 12 spines	9 rings to level of ventral sucker	3 - 4 rings	173 µm (fixed in hot formalin)	236 µm
Cercaria maritzburgensis Porter, 1938	Eye of fish	-	6 large pairs	As D. gasterostei but bladder arm not recurved or ciliated	Present	15 rings to posterior of body	-	145 - 200 µm	230 - 365 µm
Cercaria elodes Olivier, 1942	Penetrate tadpoles	Present	5 - 7 pairs (usually 6)	2[(4) + ((3) + (2))] = 18	3 groups of spines 6-18-6	9-10 rings to level of ventral sucker	several rings	238 µm	288 µm
Cercaria fennica IV Wikgren, 1956	-	Absent	5 - 7 pairs	"Identical with that of D. spathaceum"	Present	-	3 - 4 rings	-	-

TABLE 14: Localisation of metacercariae of Apatemon spp. in various freshwater fish

Species of fish	Source	Number Examined	Location of Metacercariae			
			Eye	Cranial Cavity + orbit	Pericardial Cavity	Body Cavity
3 Spined Stickleback (Gasterosteus aculeatus)	Trout Farm (Perthshire)	31	11	2	-	8
	Heidarvatn, Iceland	10	47	-	-	-
Rainbow Trout (Salmo gairdneri)	Trout Farm (Perthshire)	5	10	21	313	19
Perch (Perca fluviatilis)	Loch Lomond	4	70	-	-	-
Stone Loach (Noemacheilus barbatulus)	Trout Farm (Perthshire)	25	1	2	6	63

Total numbers of metacercariae recovered

TABLE 15: Dimensions of metacercariae of Apatemon gracilis from loach and trout. Measured in ventral view, in μm

		Metacercariae from trout experimentally infected 10 specimens		Metacercariae from naturally infected loach (R. Almond) 5 specimens	
Forebody	Length	380 - 543	(445)	395 - 581	(444)
	Width	209 - 271	(243)	248 - 294	(266)
Hindbody	Length	120 - 198	(152)	144 - 243	(200)
	Width	98 - 127	(112)	93 - 133	(115)
Oral Sucker	Length	67 - 80	(74)	57 - 86	(76)
	Width	68 - 82	(76)	80 - 89	(83)
Pharynx	Length	27 - 32	(28)	29 - 38	(35)
	Width	27 - 32	(29)	27 - 32	(30)
Ventral Sucker	Length	65 - 82	(74)	86 - 114	(100)
	Width	82 - 103	(95)	104 - 118	(112)

TABLE 16: Results of infection experiments using cercariae of *Diplostomum spathaceum*

Fish exposed (species and number)	Number of cercariae/fish	Number of fish infected	Number and % recovery/fish	
Minnow (6)	42	6	17.2	40.8%
Goldfish (5)	100	1	0.4	0.4%
Rainbow trout (5)	100	5	39.5	39.5%
3-spined stickleback (4)	38	4	15.8	24%
Perch (5)	100	5	4.8	4.8%

TABLE 17: Results of infection experiments using *D. gasterostei* cercariae from the Forth and Clyde Canal

Fish exposed (species and numbers)	Number of cercariae/fish	Number of fish infected	Number and % recovery/fish	
3-spined sticklebacks (5)	44	5	24.4	56%
Goldfish (5)	111	0	0	0
Minnows (5)	111	1	0.2	0.18%
Rainbow trout (4)	177	4	14.8	8.4%
Stone loach (3)	185	1	0.3	0.16%
Perch (3)	185	0	0	0

TABLE 18: Results of infection experiments using D. gasterostei cercariae from Packington, Birmingham

Fish exposed (species and numbers)	Number of cercariae/fish	Number of fish infected	Number and % recovery/fish	
3-spined stickleback (1)	80	1	49	61%
Goldfish (5)	80	0	0	0
Brown trout (5)	80	4	0.8	1.0%
Rainbow trout (5)	80	3	1.4	1.7%
Perch (5)	80	0	0	0

TABLE 19: Results of infection experiments with D. gasterostei cercariae experimentally derived from metacercariae in perch

Fish exposed (species and numbers)	Number of cercariae/fish	Number of fish infected	Number and % recovery/fish	
3-spined stickleback (2)	68	2	32.5	48%
Perch (2)	143	2	41.5	29%

TABLE 20: Results of infection experiments with cercariae of Diplostomum sp.

Fish exposed (species and numbers)	Number of cercariae/fish	Number of fish infected	Number and % recovery/fish	
3-spined stickleback (3)	42	2	2.3	5.5%
Minnow (1)	42	1	2	4.8%
Rainbow trout (3)	42	3	32	76%
Perch (3)	42	0	0	0

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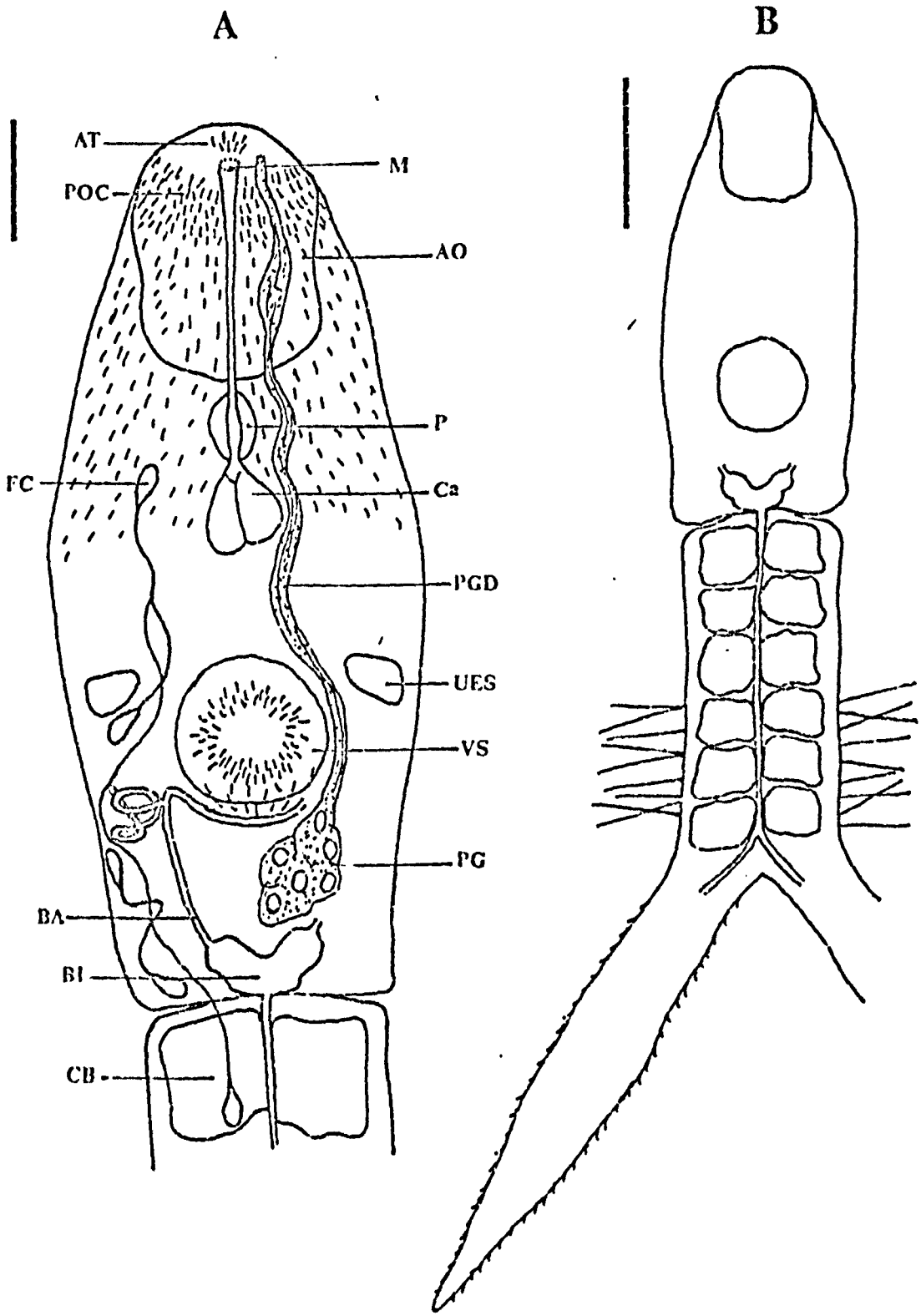
Figure 10 Diagrams of the cercaria of Apatemon gracilis

A) The body of the cercaria in ventral view. The bar represents approximately 20μm.

B) Diagram showing the relative proportions of the body, tail-stem, caudal bodies, and furcae. The bar represents approximately 50μm.

key to abbreviations on page xi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facing page 125

Figure 11 Phase-contrast photomicrograph of the body of the cercaria of Apatemon gracilis. Note caeca, ventral sucker, penetration glands and their nuclei (one arrowed) and the bladder. Bar represents approximately 25μm.

key to abbreviations on page xi

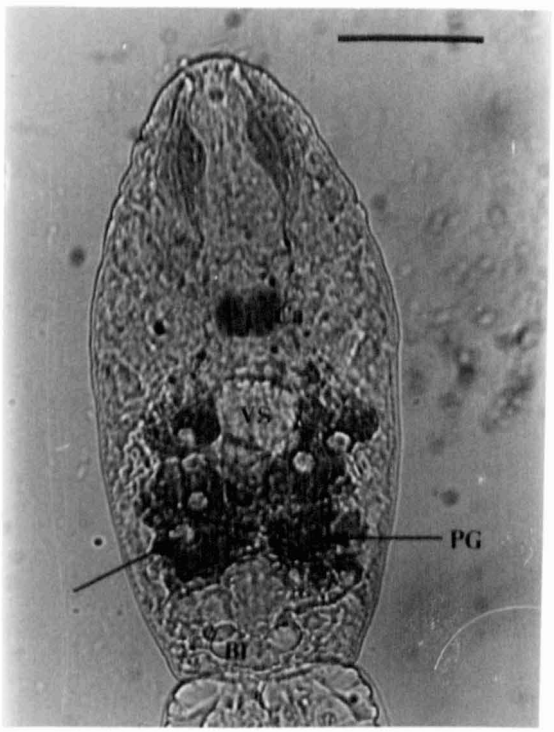
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Figure 12 Diagrams of the body of the cercaria of Diplostomum spathaceum

A) Ventral view

B) Lateral view

The bar represents approximately 20μm.

key to abbreviations on page xi

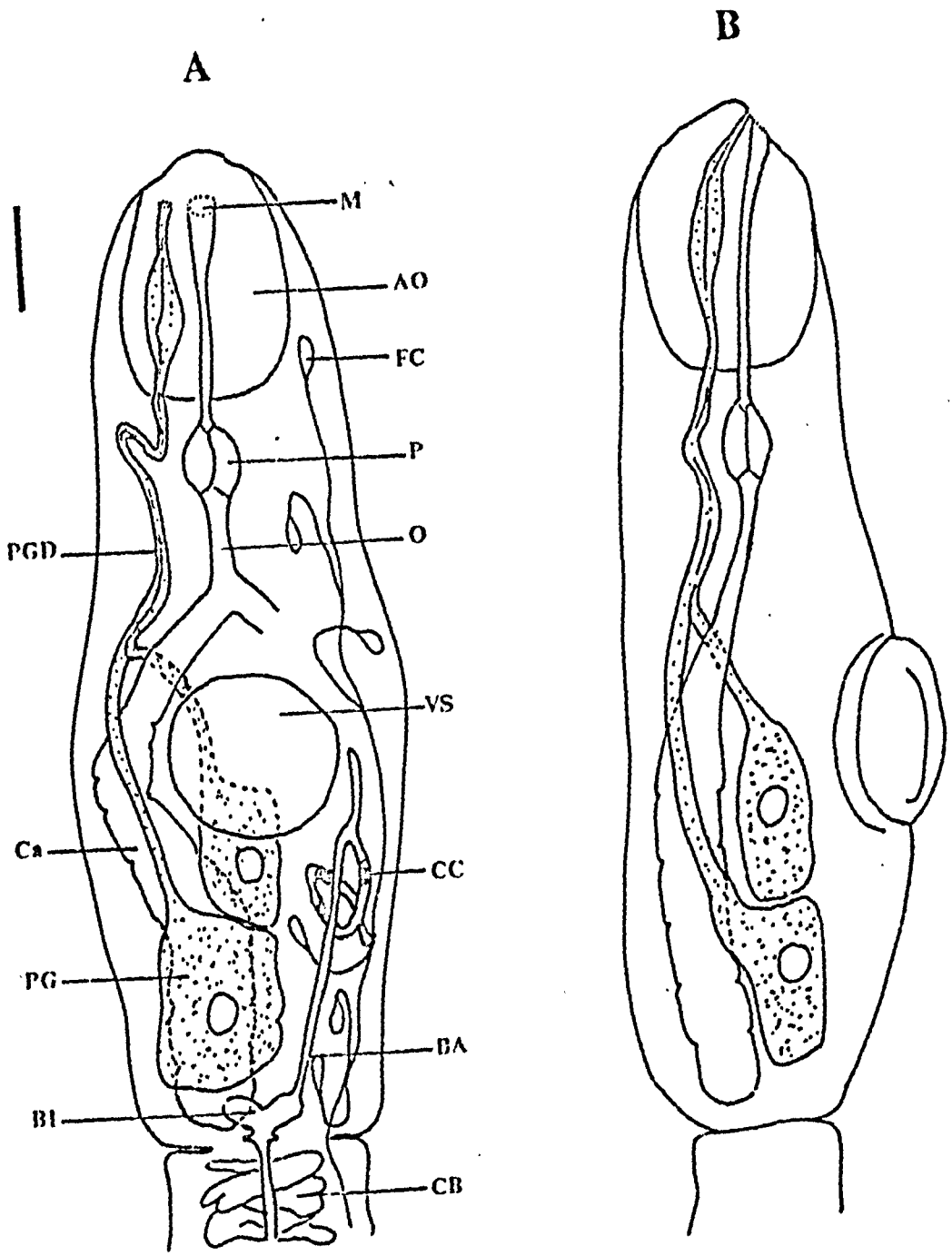
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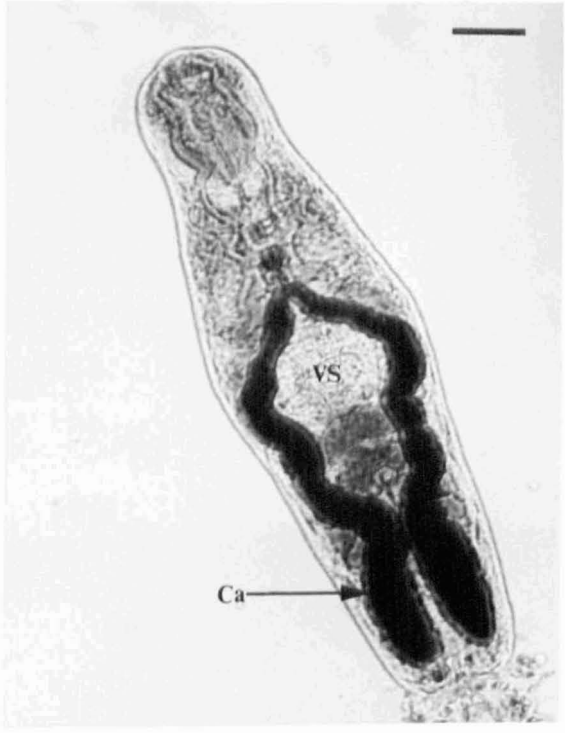
Figure 13 Photomicrograph of the body of the cercaria of Diplostomum sp. stained with neutral red. Note the gut caeca and the ventral sucker. Bar represents approximately 25μm.

Figure 14 phase-contrast photomicrograph of the body of the cercaria of Diplostomum sp. Note the anterior organ, penetration gland ducts, pharynx, oesophagus, and the ventral sucker. Bar represents approximately 25μm.

key to abbreviations on page xi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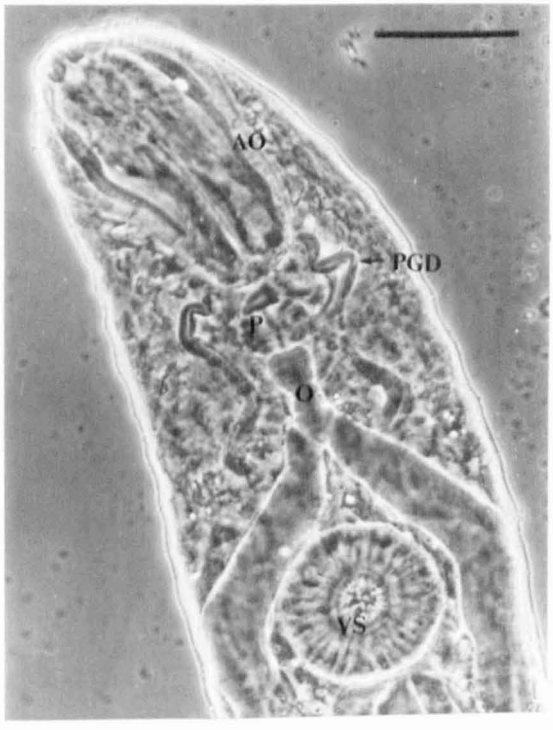
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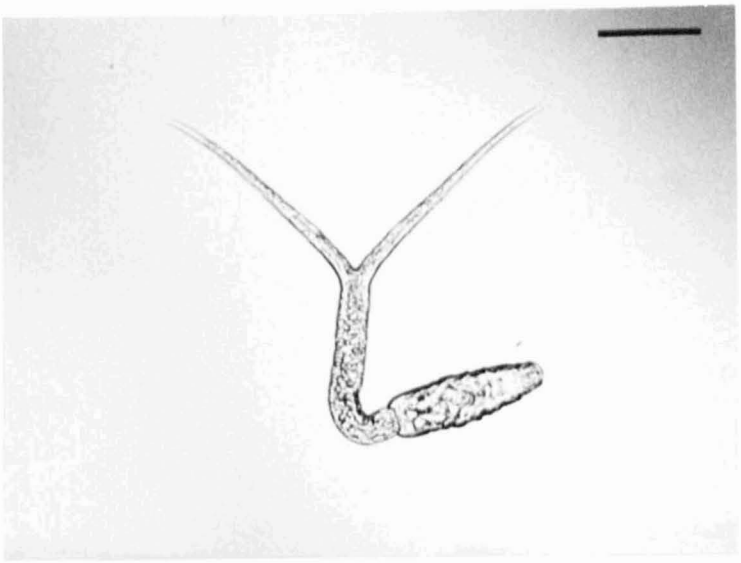
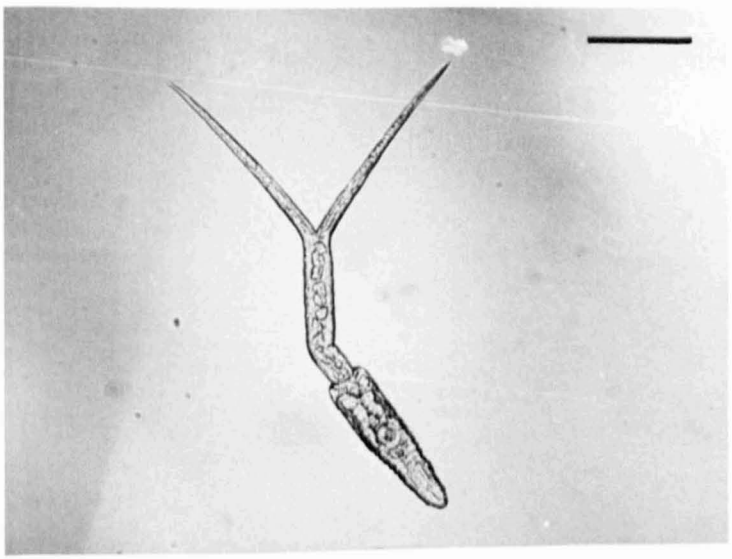
facing page 128

Figure 15 Photomicrograph of the cercaria of Diplostomum spathaceum in its typical resting posture. Ventral view. Bar represents approximately 100μm.

Figure 16 Photomicrograph of the cercaria of Diplostomum sp. in its typical resting posture. Ventral view. Bar represents approximately 100μm.

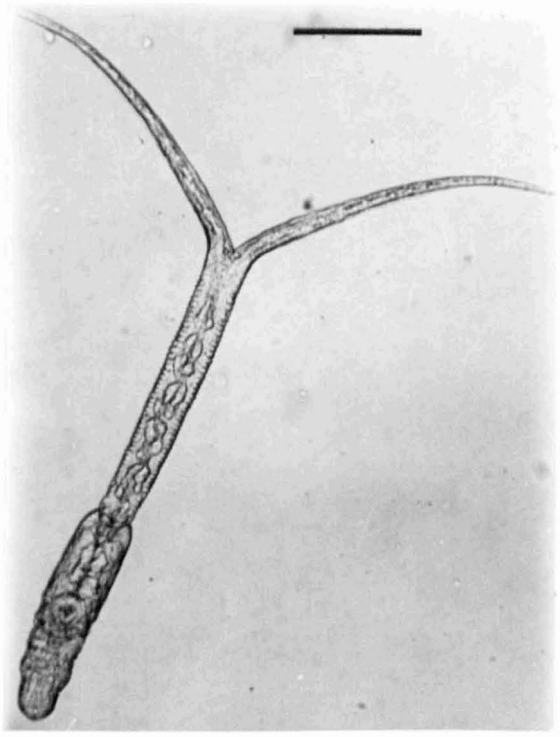




facing page 129

Figure 17 Photomicrograph of the cercaria of Diplostomum gasterostei in its typical resting posture. Ventral view. Bar represents approximately 100μm.

Figure 18 Photomicrograph of the cercaria of Diplostomum gasterostei in its typical resting posture. Lateral view. Bar represents approximately 100μm.



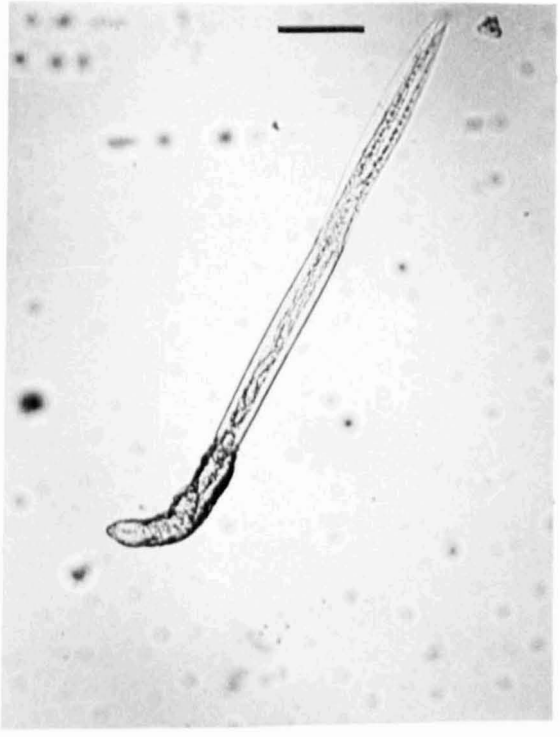


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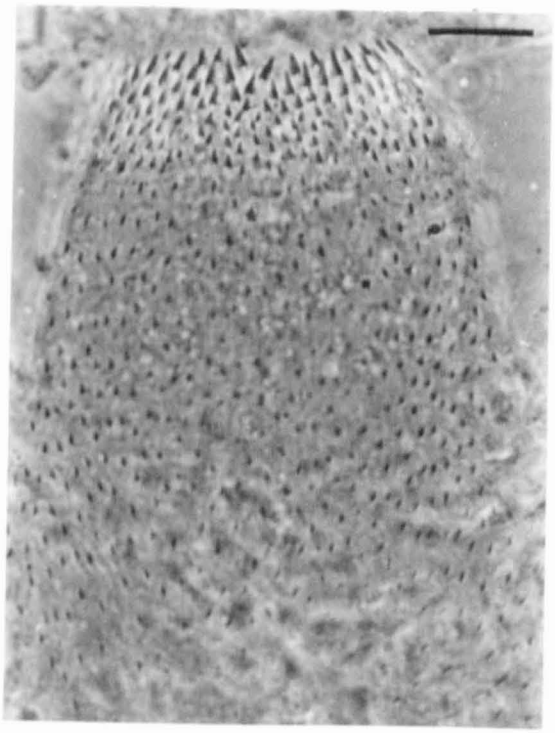
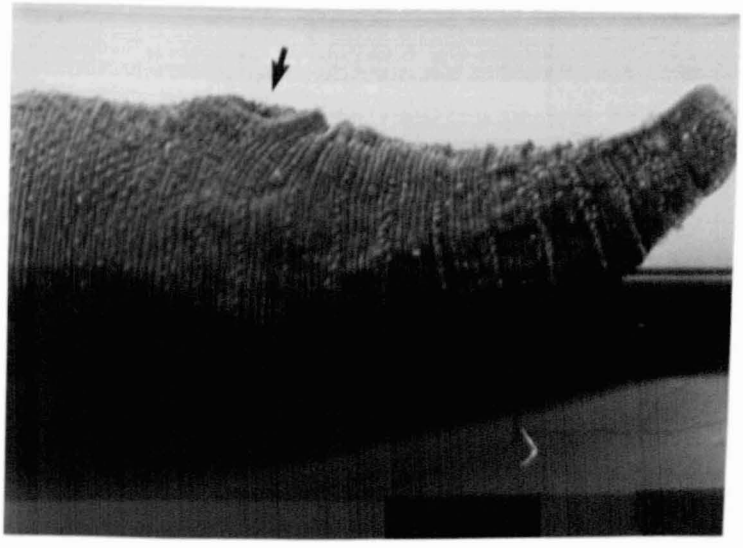
Figure 19 Phase-contrast photomicrograph of spines on the anterior half of the body of an Apatemon gracilis cercaria. Dorsal view. Anterior end of cercaria at top of figure. Apical tuft spines missing. Bar represents approximately 15μm.

Figure 20 Scanning electron micrograph of the body of a Diplostomum spathaceum cercaria. Lateral view. Note position of ventral sucker (arrowed) and arrangement of spines. Bar represents 20μm.





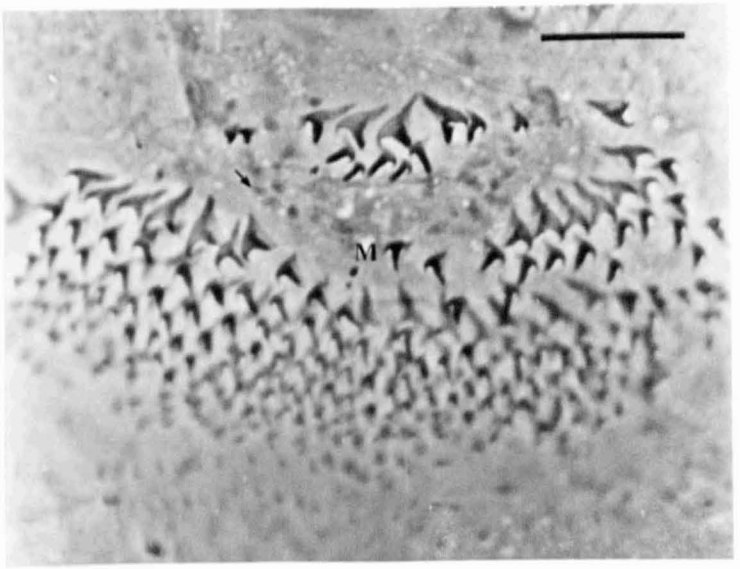
facing page 131

Figure 21 Phase-contrast photomicrograph of apical tuft and post-oral collar spines on a cercaria of Diplostomum spathaceum (derived from metacercariae in roach). Some spines of the apical tuft have been displaced. Note mouth opening surrounded by sensory papillae (faintly visible, one arrowed). Bar represents approximately 10μm.

Figure 22 Phase-contrast photomicrograph of cercaria of Diplostomum sp. Dorsal view. Note post-oral collar, and first two rings of body spines (arrowed). Bar represents approximately 25μm.

Figure 23 Scanning-electron micrograph of cercaria of Diplostomum sp. Lateral view. Note rings of spines on body and the additional spines in rings laterally. The first ring behind anterior organ is at top of the figure. Bar represents approximately 10μm.

key to abbreviations on page xi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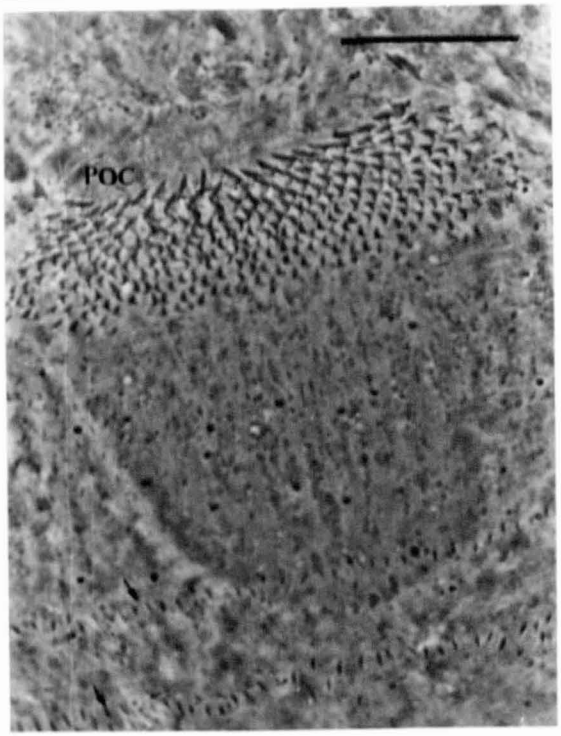
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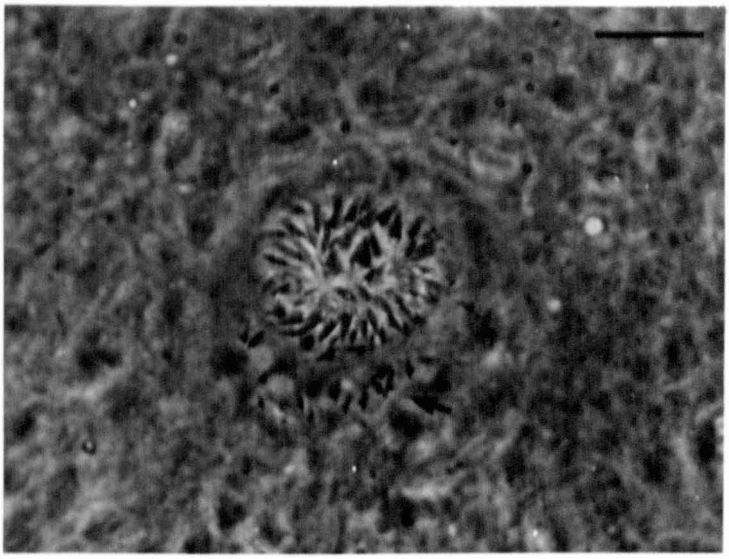
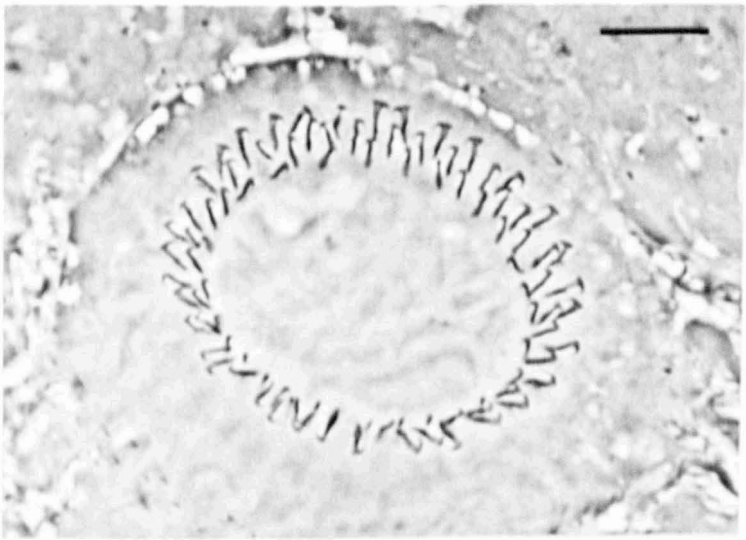
facing page 132

Figure 24 Phase-contrast photomicrograph of spines on the ventral sucker of an Apatemon gracilis cercaria. Note scattered posteriorly-directed spines (arrowed) outwith the main ventral sucker rings. Bar represents approximately 10μm.

Figure 25 Phase-contrast photomicrograph of spines on the ventral sucker of a Diplostomum gasterostei cercaria (derived from metacercariae in Loch Lomond perch). Bar represents approximately 10μm.

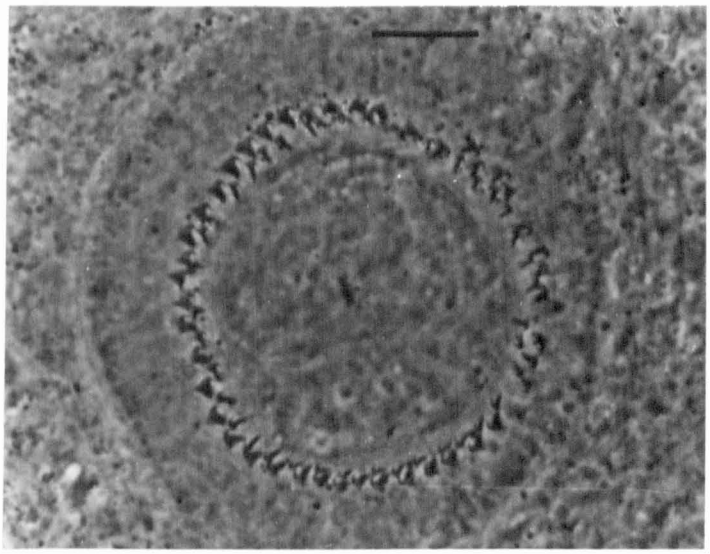


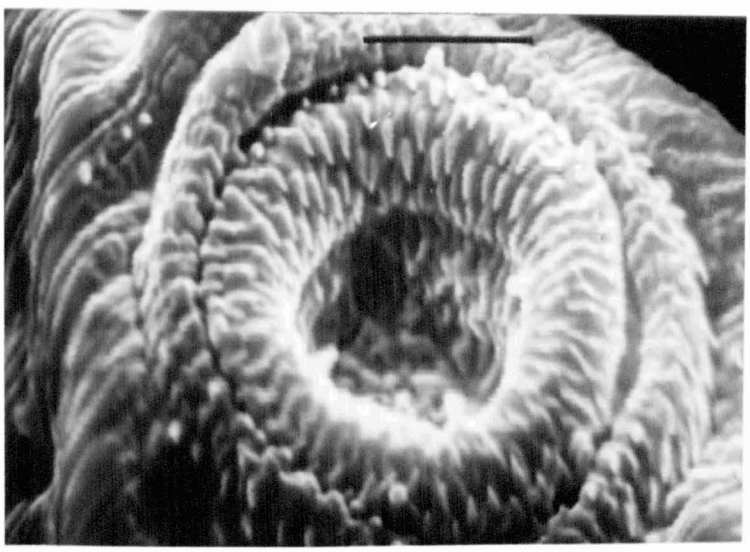


facing page 133

Figure 26 Phase-contrast photomicrograph of spines on the ventral sucker of a Diplostomum sp. cercaria. Bar represents 10μm.

Figure 27 Scanning-electron micrograph of the ventral sucker of a Diplostomum spathaceum cercaria. Note spines on anterior arc of sucker (towards top of figure) directed into its cavity. Bar represents approximately 10μm.

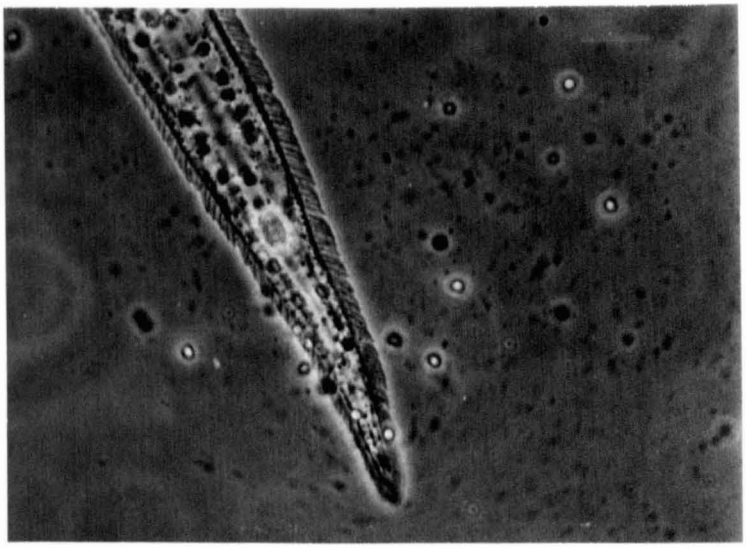




facing page 134

Figure 28 Phase-contrast photomicrograph of the furca of a Diplostomum sp. cercaria. Note fin-fold on the margins of the furca. Bar represents approximately 25μm.

Figure 29 Photomicrograph of the furca of any Diplostomum sp. cercaria, stained with silver nitrate. Note dark-staining papillae with cilia arising from them (one arrowed). Bar represents approximately 25μm.



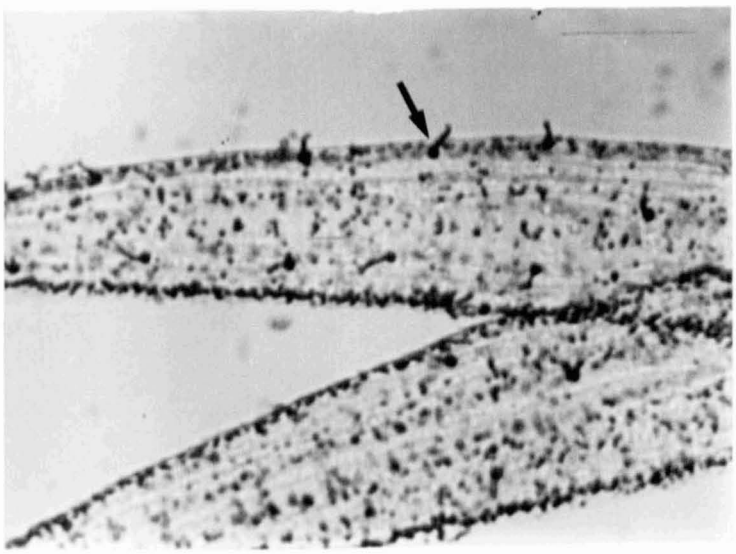


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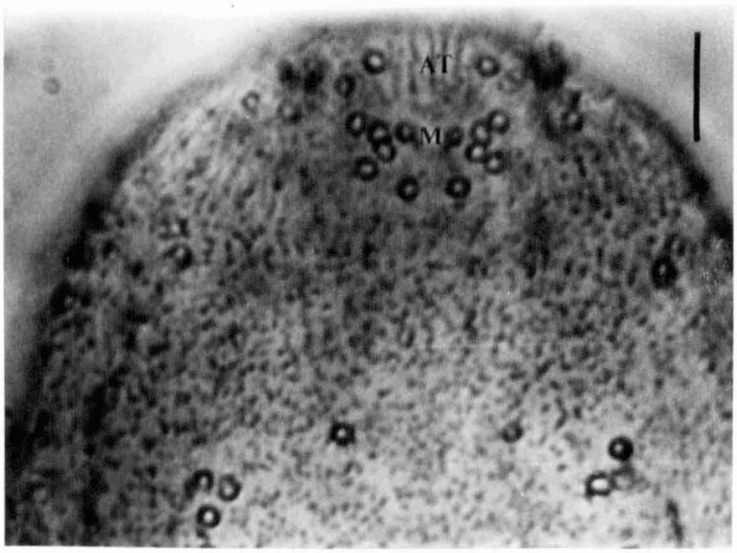
Figure 30 Photomicrograph of the anterior of the body of an Apatemon gracilis cercaria, stained with silver nitrate to show sensory papillae (appearing as dark rings). Ventral view. Note position of mouth opening and apical tuft of spines. Bar represents approximately 10μm.

Figure 31 Photomicrograph of the anterior of the body of any Diplostomum sp. cercaria, stained with silver nitrate to show sensory papillae (appearing as dark rings). Ventral view. Note position of mouth opening, and of penetration gland duct openings (arrowed). Bar represents approximately 10μm.

key to abbreviations on page xi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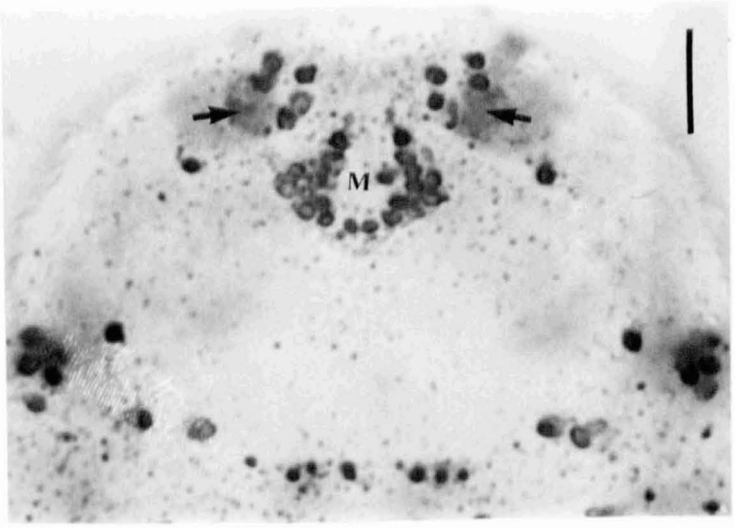
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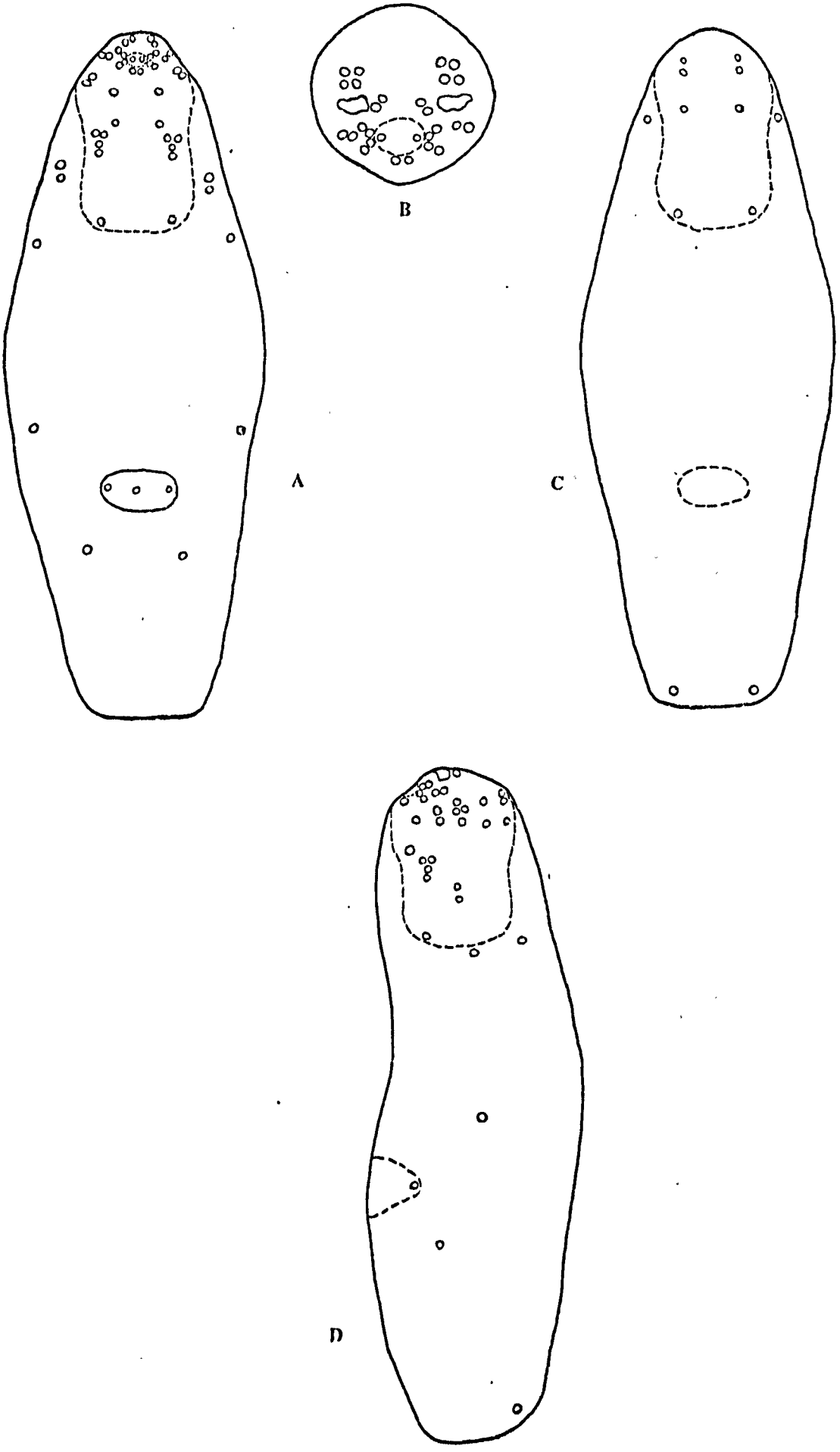
facing page 136

Figure 32 Diagrams of the arrangement of sensory papillae on the cercaria of Apatemon gracilis, stained with silver nitrate

Page 136; A) body in ventral view,
B) apical view, C) dorsal view,
D) lateral view.

Page 137; E) tail-stem in dorsal view,
F) ventral view, G) lateral view,
H) medial (inner) face of furca.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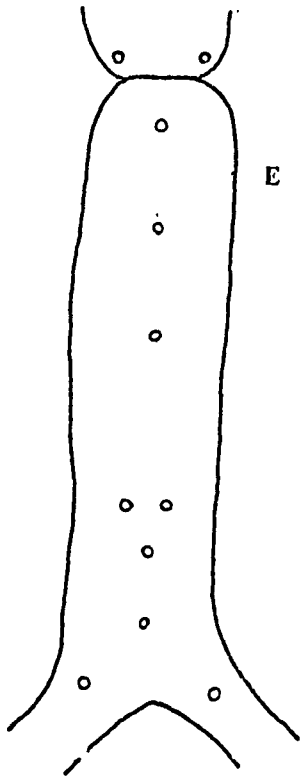
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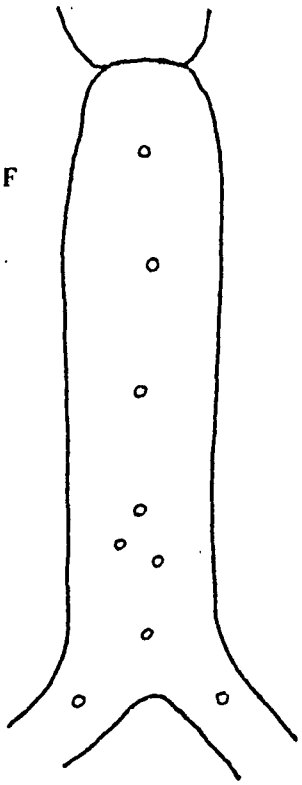
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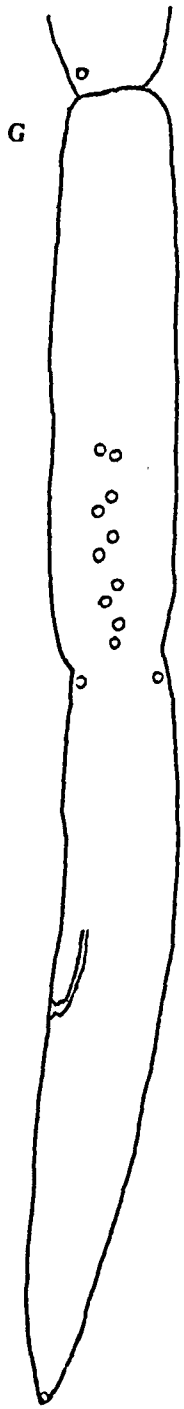
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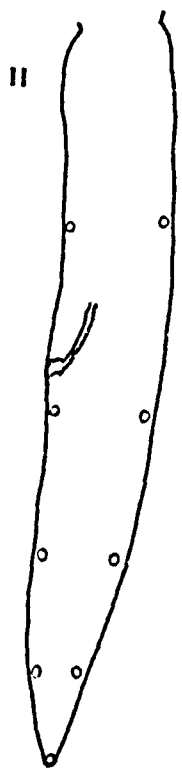
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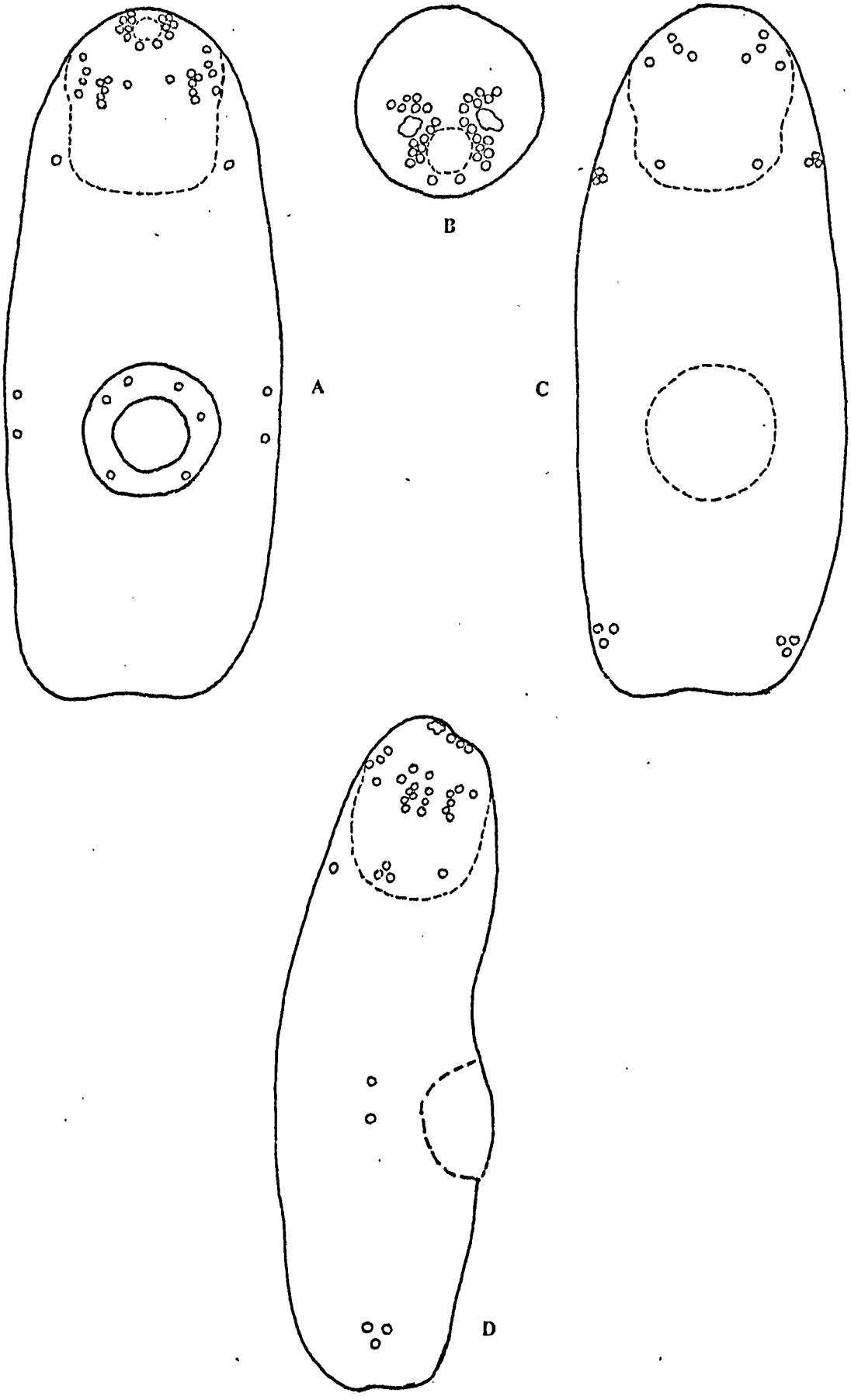
facing page 138

Figure 33 Diagrams of the arrangement of sensory papillae on the cercaria of Apatemon minor stained with silver nitrate

Page 138; A) body in ventral view
B) apical view C) dorsal view
D) lateral view

Page 139; E) tail-stem in dorsal/ventral view
F) lateral view
G) medial (inner) face of furca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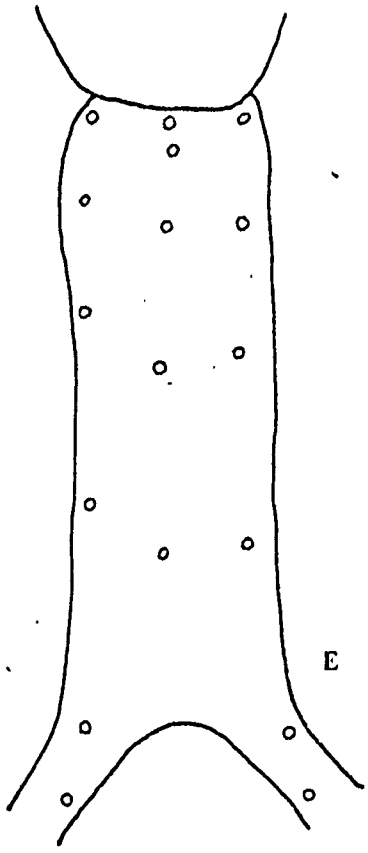

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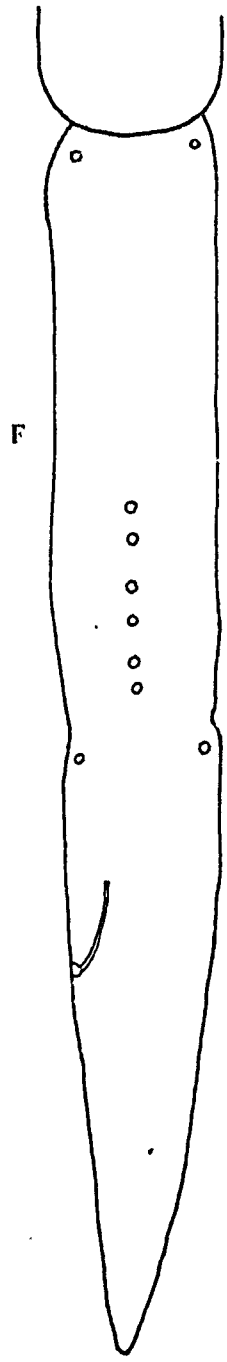

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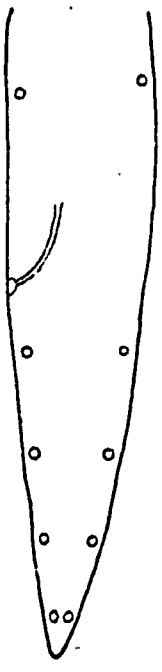

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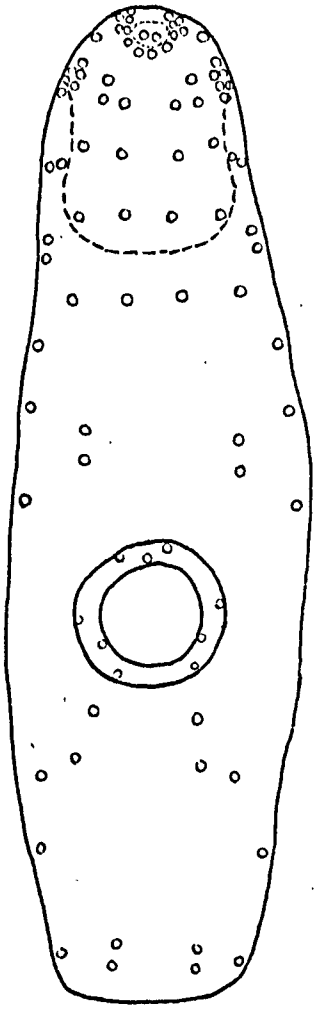
facing page 140

Figure 34 Diagrams of the arrangement of sensory papillae on the cercaria of Diplostomum spathaceum, stained with silver nitrate

Page 140; A) ventral view of body
B) apical view C) dorsal view
D) lateral view

Page 141; E) tail-stem in dorsal view
F) ventral view G) lateral view
H) medial (inner) face of furca



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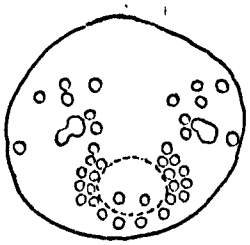

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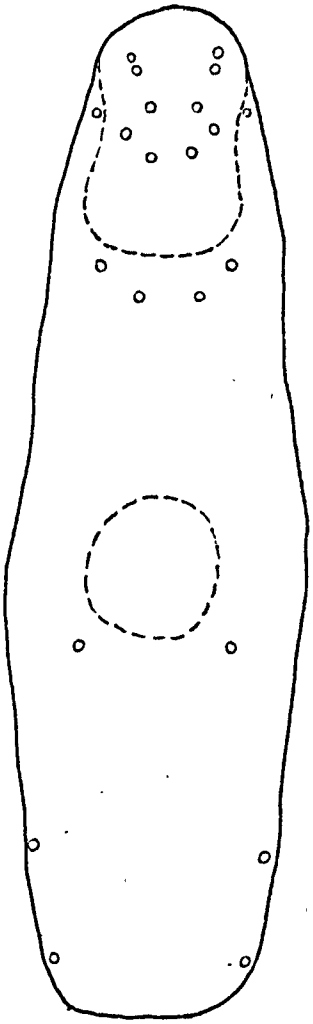

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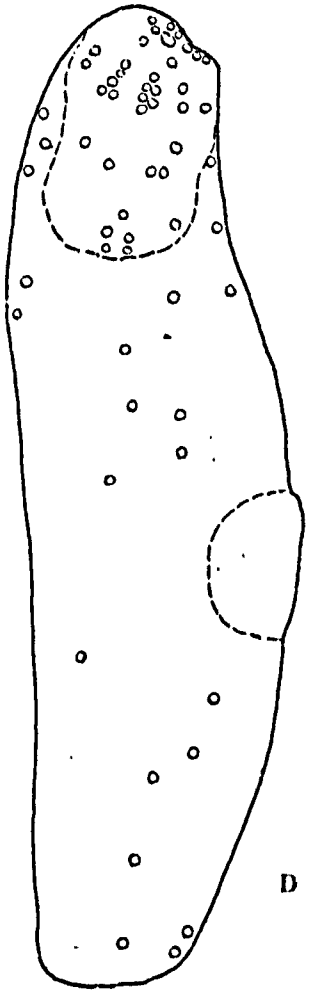

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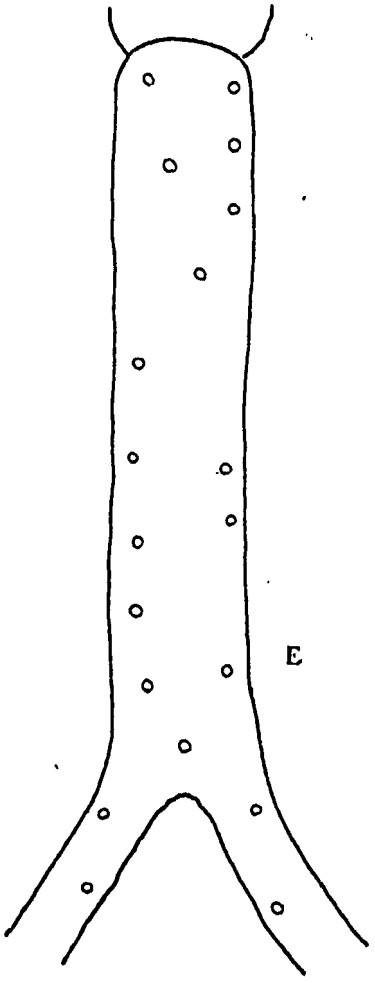
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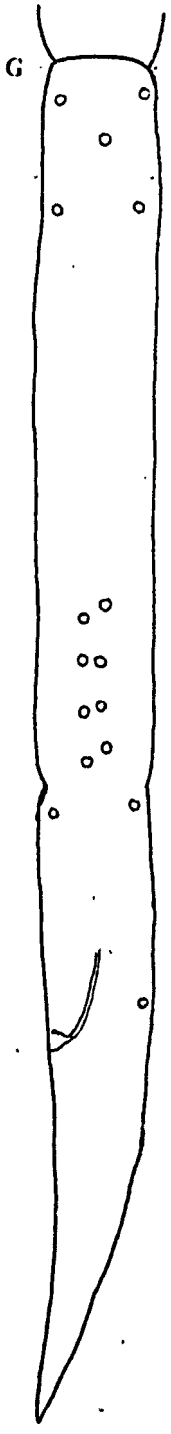
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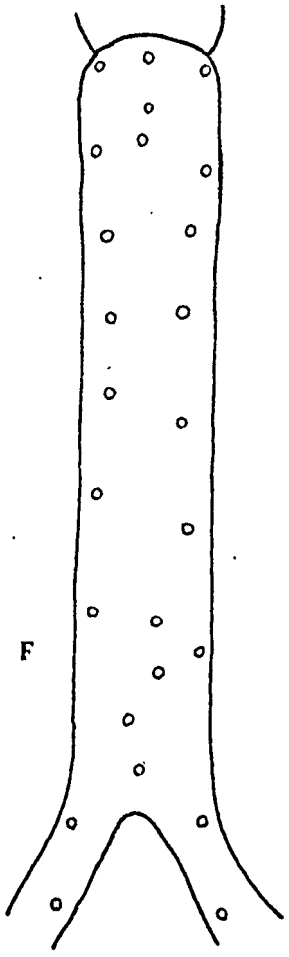
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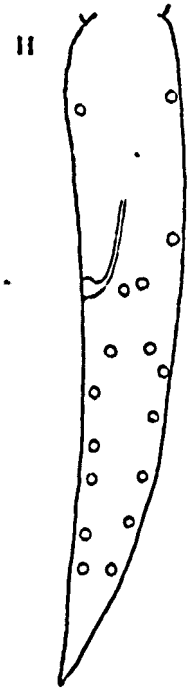
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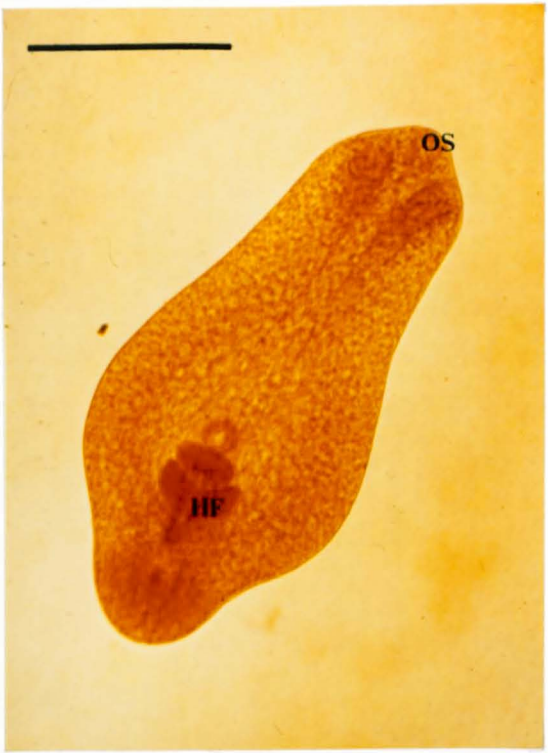
Figure 35 Photomicrograph of a pre-encystment metacercaria of Apatemon gracilis from the body cavity of an experimentally infected trout. Ventral view. Fixed in hot formol-saline and stained with Gower's carmine. Note poorly developed holdfast. Bar represents approximately 250μm.

Figure 36 Photomicrograph of a live pre-encystment metacercaria of Apatemon gracilis from the body cavity of a trout. Note presence of many lipid droplets. Bar represents approximately 250μm.

key to abbreviations on page xi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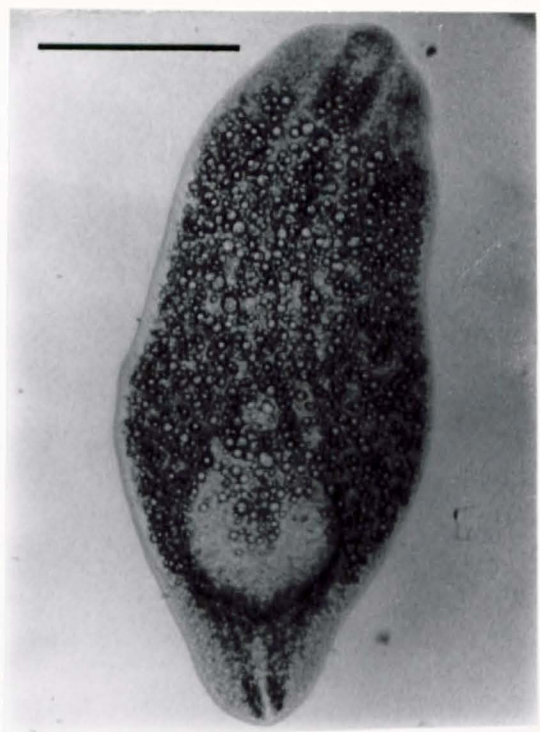

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Figure 37 Photomicrograph of an excysted metacercaria of Apatemon gracilis. Ventral view. Stained Gower's carmine. Note oral sucker, deep concavity of forebody containing the ventral sucker and holdfast, and the genital rudiments. Bar represents approximately 100μm.

Figure 38 As Figure 37, lateral view.

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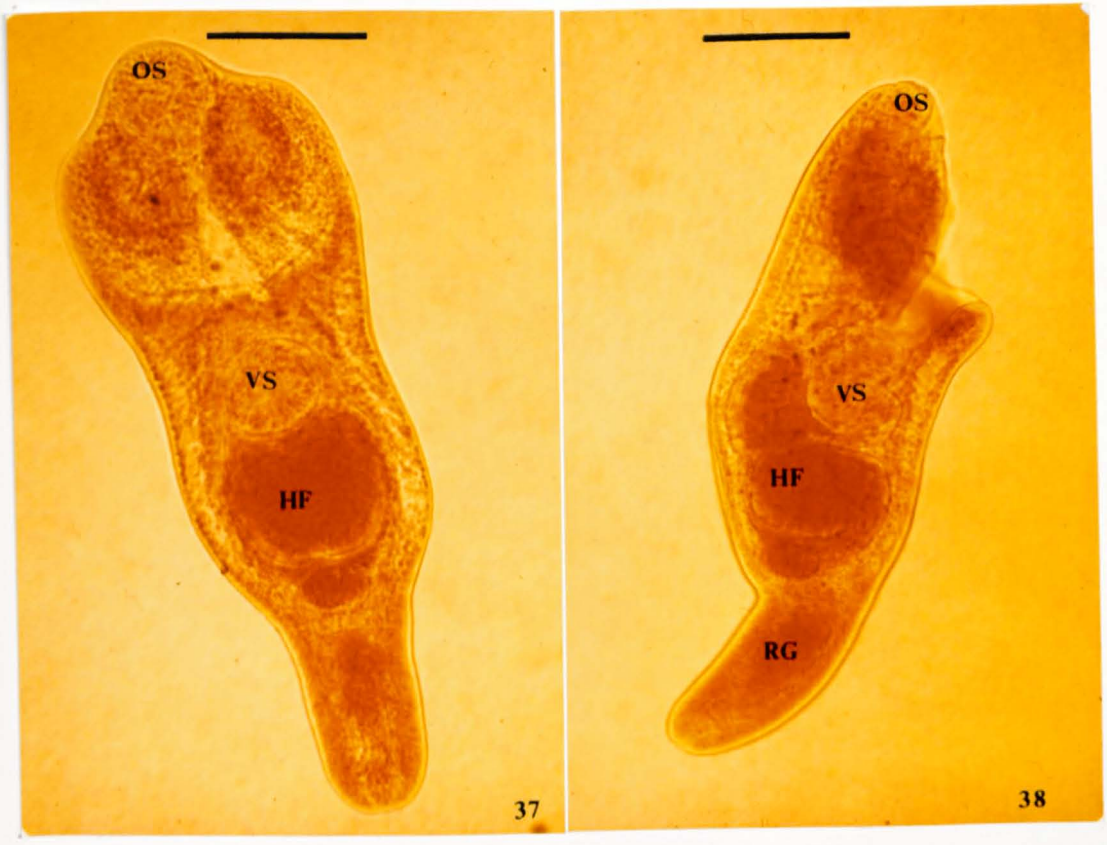
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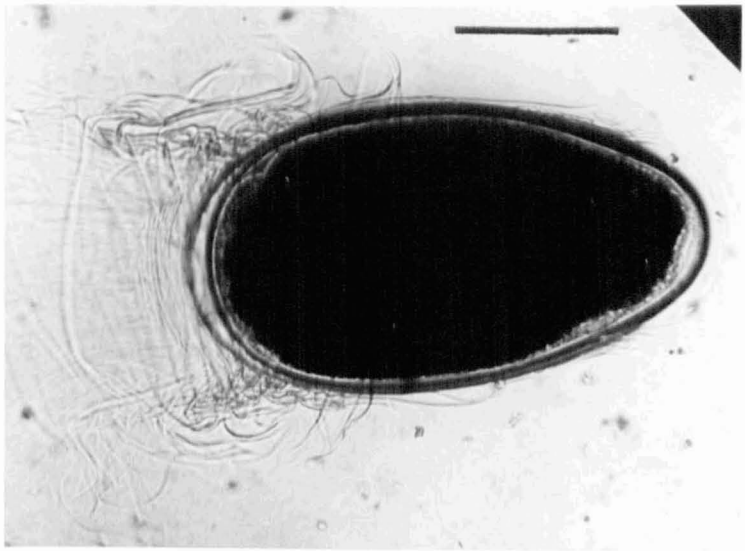
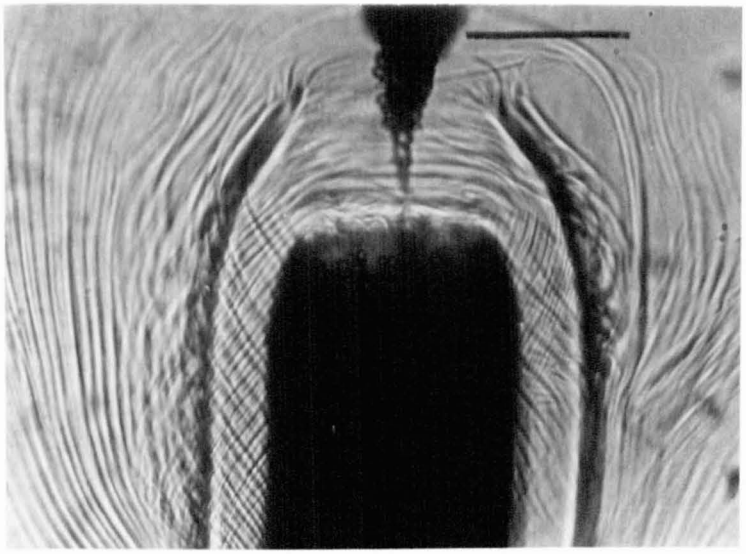
Figure 39 Photomicrograph of a cyst of Apatemon gracilis during incubation in trypsin. Note cyst layers peeling back from the pole where rupture, and expulsion of the metacercaria, will occur. Bar represents approximately 200μm.

Figure 40 Photomicrograph of an empty cyst of Apatemon gracilis after expulsion of its worm. Note lipid droplets emerging through the ruptured pole, sloughing of cyst wall lamellae, and diagonal folds in the cyst wall. Bar represents approximately 100μm.

Figure 41 Photomicrograph of two Apatemon gracilis cysts in trypsin. The cyst on the left contains a worm. Note bulge at its pole (arrowed) just prior to rupture. Cyst on the right is empty. Note smaller size of its central cavity relative to the cyst on left. Bar represents approximately 200μm.





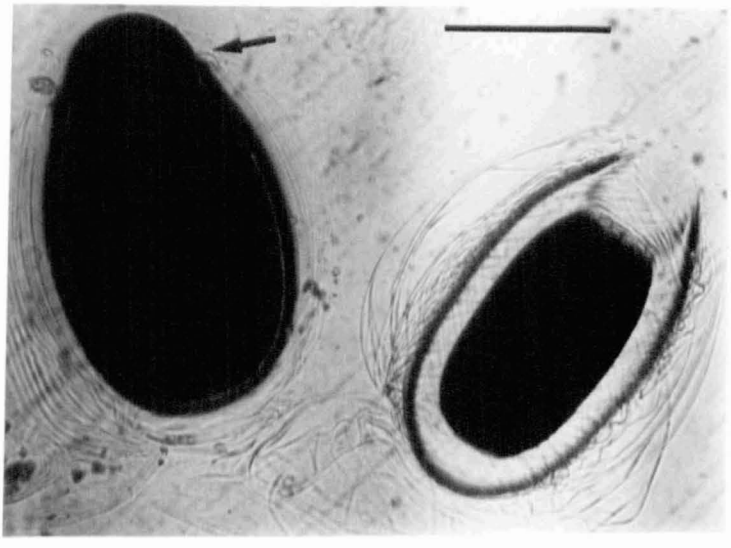


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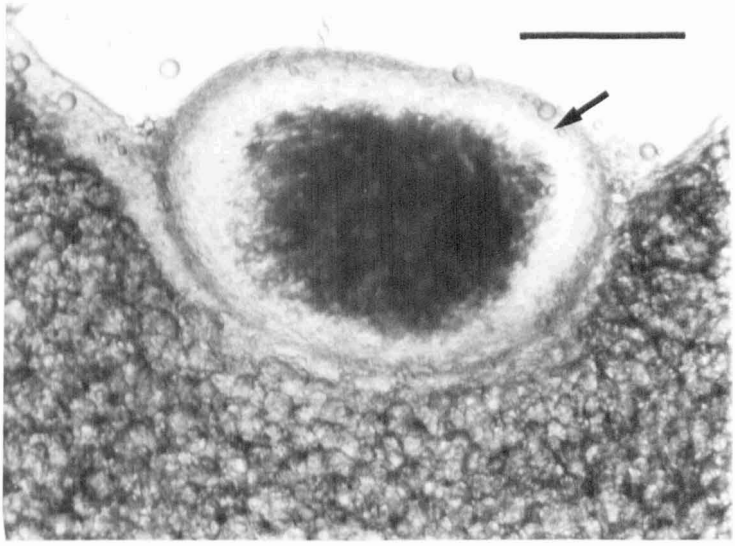
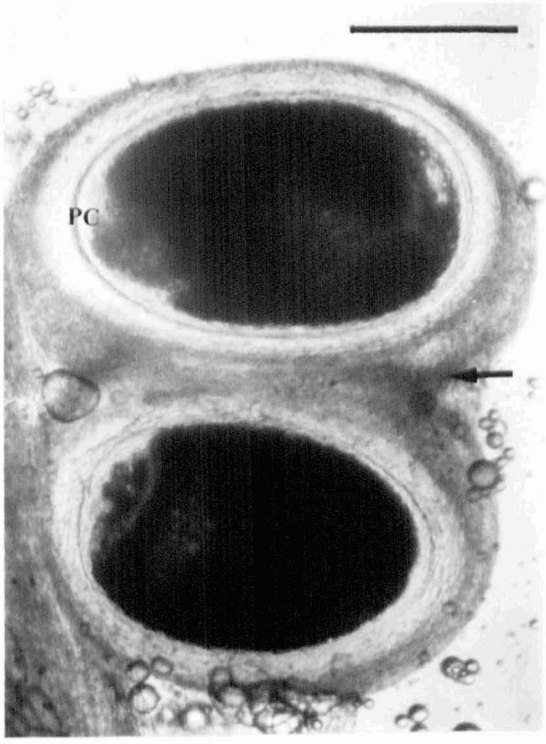
Figure 42 Photomicrograph of a dead, encapsulated, and apparently unencysted metacercaria of Apatemon gracilis on the viscera of a trout. Note outer host capsule (arrowed), and parasite (dark inner mass). Bar represents approximately 200μm.

Figure 43 Photomicrograph of two normal cysts of Apatemon gracilis on the viscera of a trout. Note host capsule (arrowed) and parasite cyst. Bar represents approximately 200μm.

Figure 44 Photomicrograph of an encapsulated Apatemon gracilis cyst from the vitreous humour of a perch eye. Note fibrous host capsule (arrowed) and enclosed cyst. Bar represents approximately 200μm.

key to abbreviations on page xi

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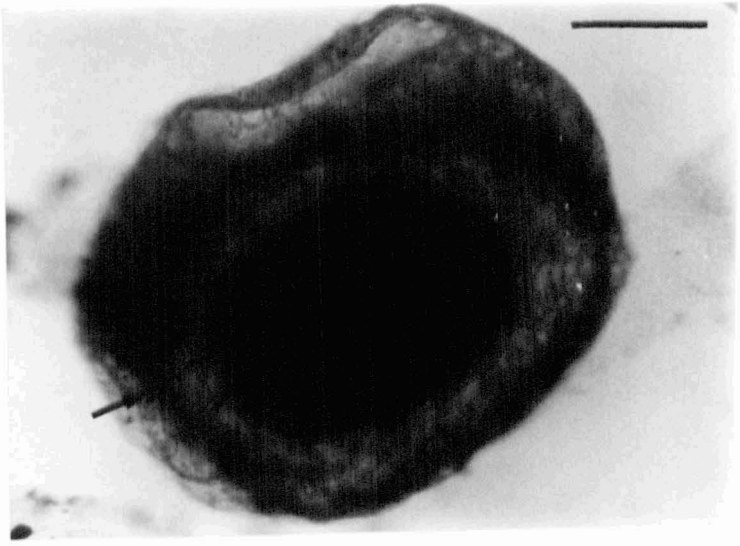

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Figure 45 Diagram of the gross morphology of a metacercaria of the subgenus Diplostomum. Ventral view. The main ducts of the reserve excretory system, and a few calcareous bodies are shown. The connection between the primary and reserve excretory systems is arrowed. The bar represents approximately 50 μm.

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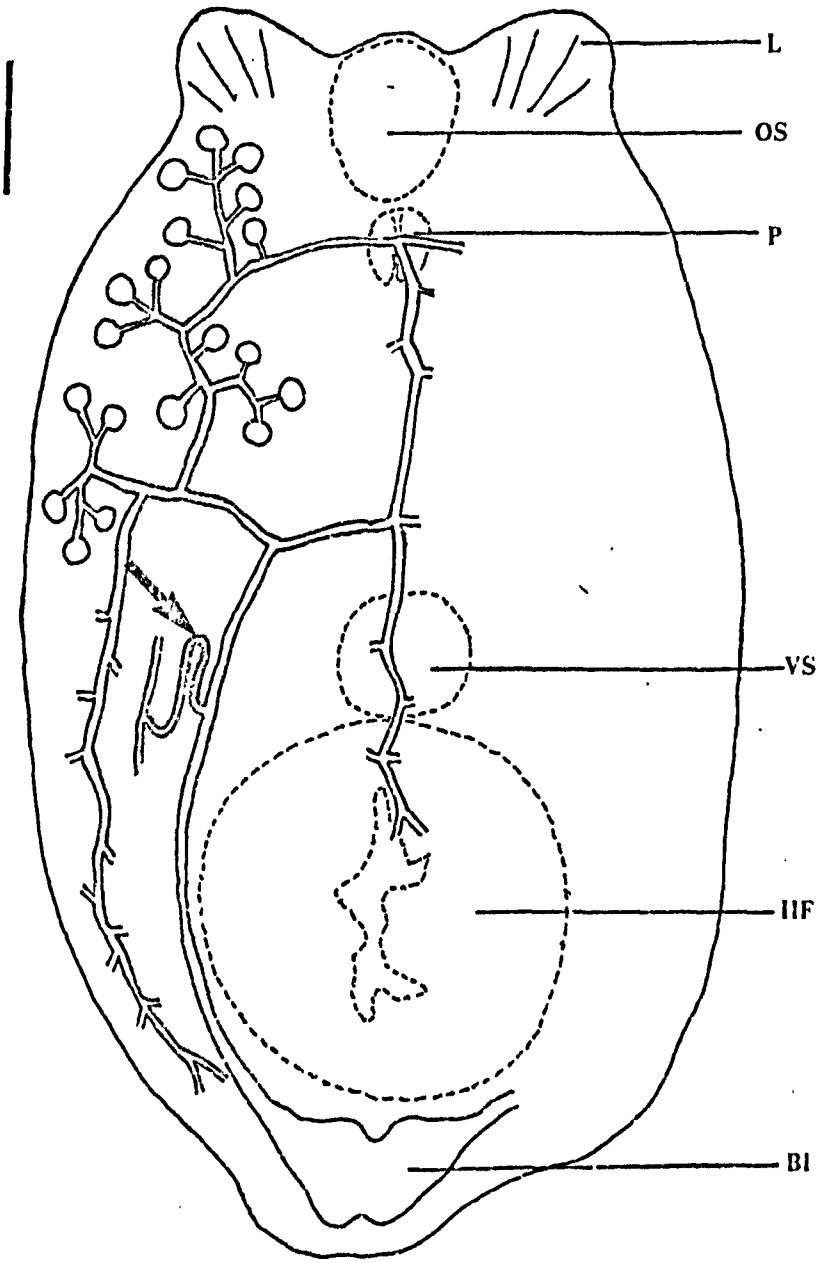
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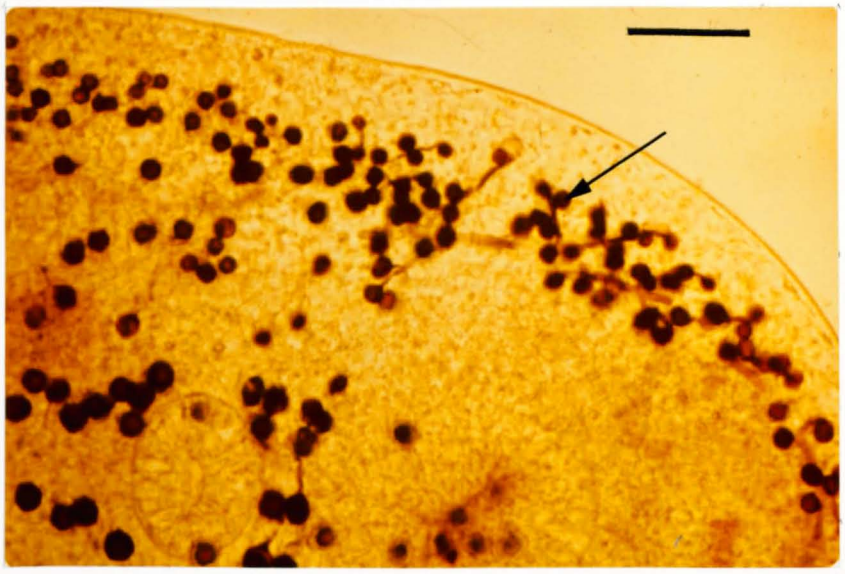
Figure 46 Photomicrograph of a Diplostomum sp. metacercaria stained with alizarin red S. Note red-staining calcareous bodies (one arrowed). Bar represents approximately 25μm.

Figure 47 Photomicrograph of a frozen section of an Apatemon gracilis metacercaria stained with Sudan black V. Note large lipid droplets (one arrowed). Bar represents approximately 100μm.



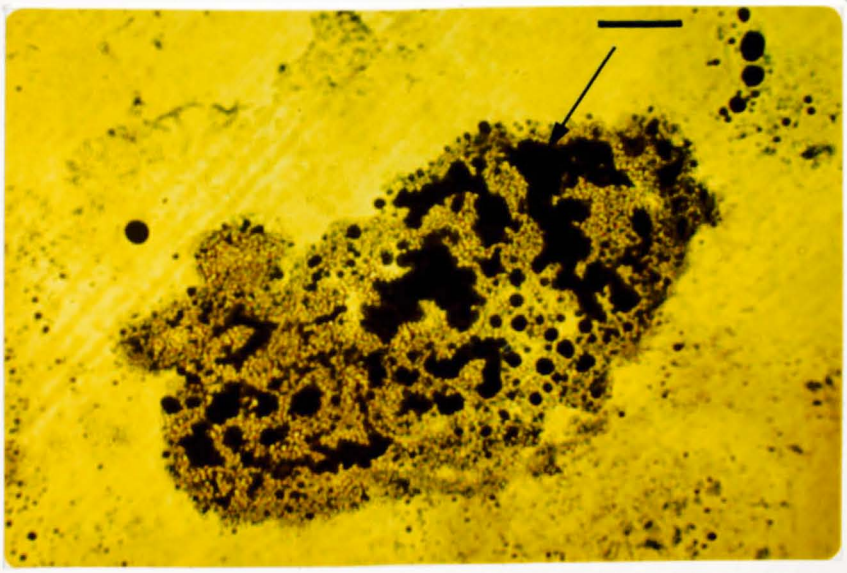


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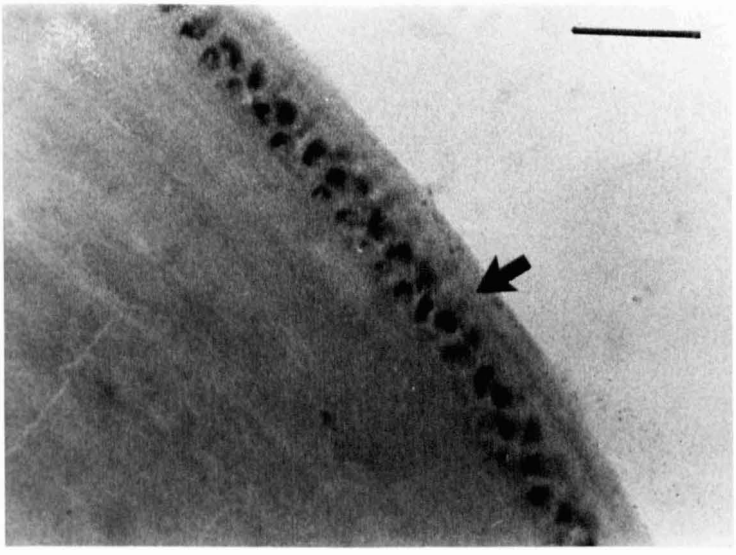
Figure 48 Photomicrograph of a section of normal trout lens stained with haematoxylin and eosin. Note healthy lens epithelium (arrowed). Bar represents approximately 50μm.

Figure 49 Photomicrograph of a section of trout lens infected with Diplostomum spp. metacercariae, and stained with haematoxylin and eosin. Note invasive proliferation of epithelial cells into lens cortex (arrowed). Bar represents approximately 50μm.



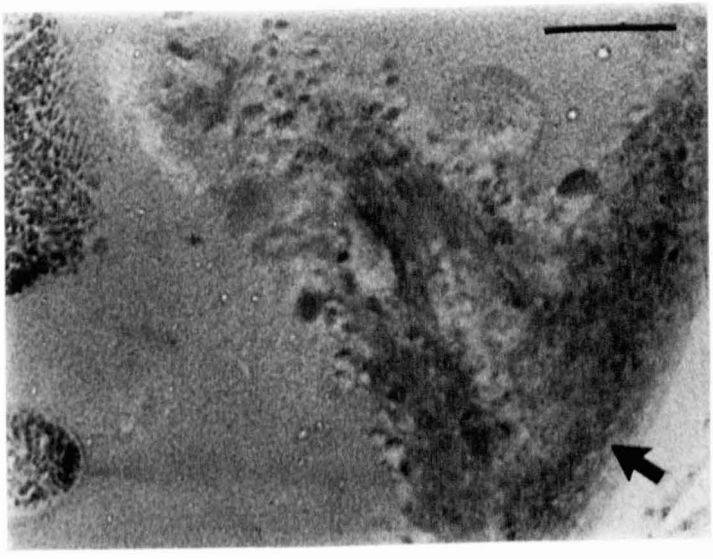


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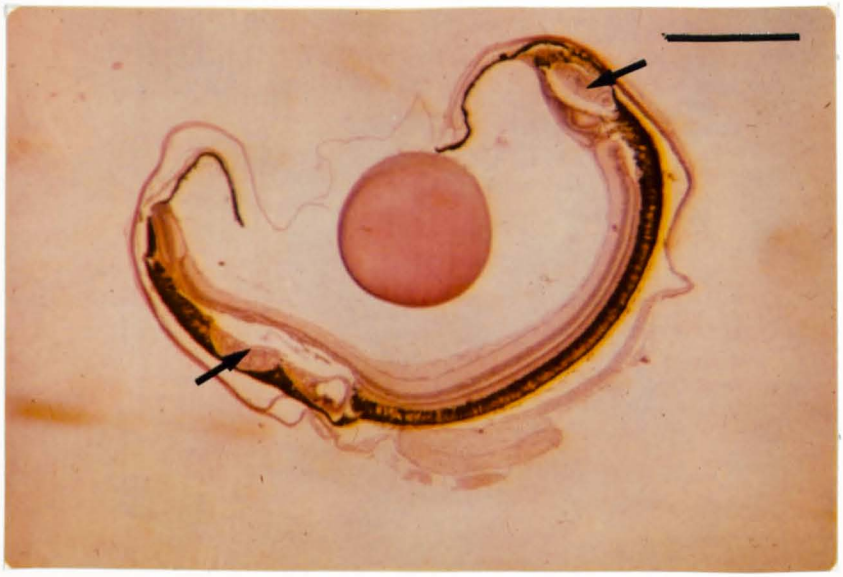
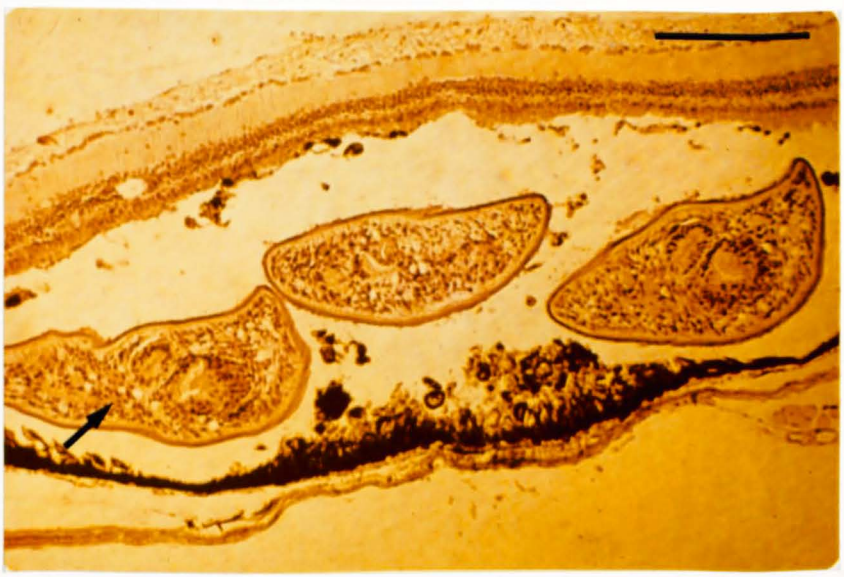
Figure 50 Photomicrograph of a section of a stickleback eye stained with haematoxylin and eosin. Note metacercariae of Diplostomum gasterostei in retina (arrowed). Bar represents approximately 250μm.

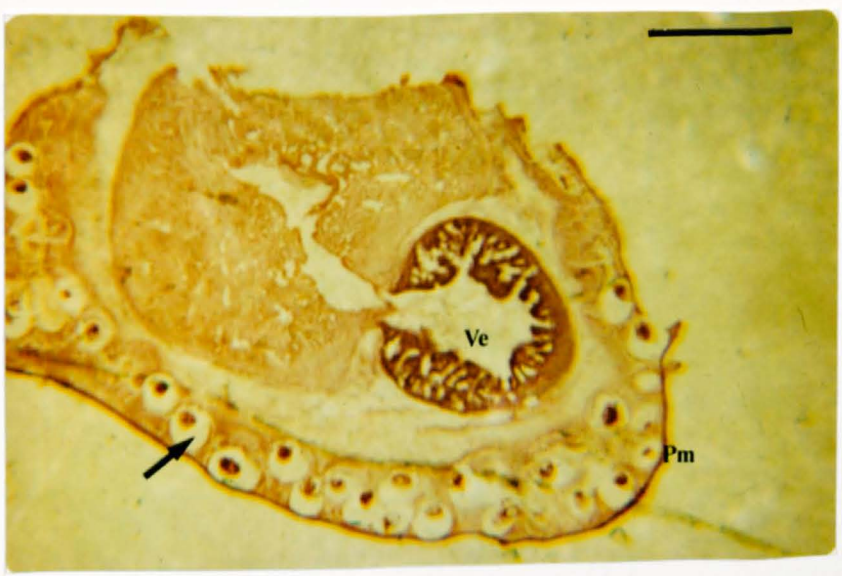
Figure 51 Photomicrograph of a section of stickleback retina stained with haematoxylin and eosin. Note Diplostomum gasterostei metacercariae (one arrowed). Bar represents approximately 100μm.

Figure 52 Photomicrograph of a section of trout heart and pericardium stained with haematoxylin and eosin. Note presence of many Apatemon gracilis cysts (one arrowed) within the pericardial cavity. Bar represents approximately 2mm.

key to abbreviations on page xi



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CHAPTER 3

EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF STRIGEOID EGGS

INTRODUCTION

The eggs of strigeoids, as of many other trematodes and pseudophyllidean cestodes, are operculate and develop in water. Although there are numerous accounts of the development of such eggs (see Smyth, 1966 pp 66-71, and Erasmus, 1972 pp 45-50), experimental studies of environmental factors influencing development and hatching of operculate trematode eggs appear to be limited to Fasciola hepatica (see Rowcliffe and Ollerenshaw, 1960).

During this study, eggs of Diplostomum spathaceum were obtained from experimentally infected black-headed gulls. The opportunity was therefore taken of carrying out experimental studies on the factors affecting development and hatching of these strigeoid eggs. It was hoped that an understanding of these would help explain the pattern of trematode infections in snails, to be discussed in Chapter 4.

D. spathaceum is reported from numerous localities, presumably differing greatly in their water chemistry. During this study, infected snails were found in fast flowing highland streams, as well as in static waters of high conductivity (see page 51). Eggs will be faced with different conditions according to where they are deposited. In highland streams they may face prolonged periods of low temperatures. In stagnant conditions

oxygen depletion may occur, eggs may be buried in sediment and encounter toxic dissolved substances, or turbidity may lower incident light.

MATERIALS AND METHODS

Eggs of Diplostomum spathaceum used here were obtained by sieving the faeces of black-headed gulls fed eyes of infected roach from the Forth and Clyde Canal. For the collection of their faeces, gulls were placed in a cage with a grid bottom over a tray of water. Faecal pellets were broken up by vigorous shaking in a jar of water. The resulting slurry was poured through a nest of sieves (Endecotts (test sieves) Ltd. London) and washed with clean water. D. spathaceum eggs pass through an 88μm aperture sieve, but are retained in a 37μm sieve. Eggs in washings from the 37μm sieve were further cleaned by washing and sedimenting, after the method of Orr and Hopkins (1969).

The development of a number of eggs was followed. Individual eggs were placed in a tiny droplet of water on a coverslip. This was inverted over a cavity slide, sealed with vaseline, and could then be observed with the high power objectives of a Wild M-20 microscope.

For experimental work, eggs from freshly shed faeces (3-12 hours) were used. After sieving and washing, eggs were taken up in a drawn-out Pasteur pipette and transferred to a solid watch-glass of about 3 ml capacity. In this way, virtually all contaminants, such as uric acid crystals, were eliminated. For each experimental or control situation, a batch of four solid watch-glasses was

used, each watch-glass containing 100 eggs. Crystamycin (Glaxo Laboratories Ltd.) was added to all incubation media used to give 100 units of penicillin G and 0.1 mg of streptomycin sulphate per ml of medium. This was to inhibit bacterial growth in the watch-glasses.

To minimise evaporation from watch-glasses during incubation, each was covered with a square of glass. Several watch-glasses were placed together in a crystallising dish containing a few mls of water (to maintain high internal humidity), and covered with silver foil. Dishes were then placed in the appropriate incubator.

With the exception of those incubated in total darkness, or in anaerobic conditions, each watch-glass was examined at intervals using a Wild M-5 dissecting microscope. Eggs in which the eyespots of the miracidium were visible, or which had hatched, were counted. Hatched eggs, which could be recognised by their open operculum with remnants of the vitelline membrane attached, were removed with a drawn-out Pasteur pipette after each counting. All eggs which had hatched by the end of an experiment were classed as viable. In the case of dark or anaerobic incubations, which were generally examined before all eggs had hatched, fully embryonated eggs were also classed as viable. The day on which an experiment was set up was counted as day 0.

Experiments were set up to test a number of factors which might influence development and hatching of D. spathaceum eggs, and which might vary in natural waters. The experimental regimes are summarised below.

1) Development time of eggs at different temperatures.

Eggs were incubated in distilled water at 40°, 30°,

25°, 20°, 15°, and 10°C in normal or cooled incubators as appropriate.

2) Ability of eggs to hatch in dark.

Watch glasses containing eggs in distilled water were incubated in a light-proof container at 25°c. Batches of four dishes were removed at intervals in a darkroom, the remainder being returned to the incubator. Formalin was added to dishes removed for examination to kill any miracidia which otherwise might hatch in the light before examination. A control group was kept in the same incubator and examined at intervals.

3) Ability of eggs to develop and hatch in low concentrations of oxygen, or in the presence of inhibitors of aerobic respiration.

Difficulty was experienced in setting up anaerobic conditions such that oxygen concentration and egg development could be monitored. Early attempts included maintaining eggs in boiled water under a layer of oil, in a water bath under a gas manifold through which a mixture of oxygen-free nitrogen (95%) and carbon dioxide (5%) were passed, and in tubes of water through which the same gases were bubbled. In the first two cases, eggs developed and hatched, but in the last, they did not. However, design of these experiments was considered faulty, and reliance was only placed on results obtained when eggs were maintained in McIntosh-Fildes anaerobic jars. Four watch-glasses were placed in each of three such jars. Two jars were evacuated then refilled with the oxygen-free nitrogen-carbon dioxide mixture three times in day 0, and twice further on day 2. The third

jar, evacuated in the same way but refilled with air, acted as a control. All jars were kept at 25°C and opened for examination on day 15.

Batches of eggs were incubated separately in two commonly used inhibitors of aerobic respiration, potassium cyanide and malonic acid. The former was used as a 1.0 mM solution, and the latter as a 10 mM solution (concentrations recommended by Dawson et al., 1969 pp 385 and 89). Cyanide was buffered to pH 7.0 in a 10mM phosphate buffer (Dawson et al., 1969 p 489), and malonic acid neutralised to pH 7.0 with sodium hydroxide. Control batches were incubated in distilled water, and in phosphate buffer at pH 7.0.

In one experiment, eggs were allowed to develop in distilled water for 7 days before being transferred to a cyanide solution. All incubations were at 25°C.

4) Development and hatching of eggs in saline conditions.

In preliminary experiments, embryonated eggs were placed in solutions of several concentrations of sodium chloride. Plasmolysis of the egg contents occurred in 200mM but not in 150mM saline. The latter is approximately equivalent in osmotic pressure to the contents of the duck small intestine (Crompton and Edmonds, 1969) and was chosen as the highest concentration to be used. Batches were also incubated in two lower concentrations, 50mM and 10mM sodium chloride. Controls in distilled water and all experimental batches were incubated at 25°C.

5) Development of eggs at different pH values.

Batches of eggs were incubated at 25°C in distilled water buffered to pH values of 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. The buffers used for the first trial were;

pH	Buffer	Concentration
4.0, 5.0	sodium acetate / acetic acid	10 mM
6.0, 7.0, 8.0	phosphate buffer	10 mM
9.0	glycine / sodium hydroxide	10 mM

In a second trial, citric acid / phosphate buffers were used at 10 mM concentrations for pH 4.0 and 5.0. Other buffers were as above. All buffers were used as recommended by Dawson et al. (1969). Controls were incubated in distilled water at 25°C.

6) Survival of eggs for protracted periods at low temperatures.

Eggs from freshly deposited faeces were incubated at about 4°C in a refrigerator. At intervals of about two months, several watch-glasses were removed to an incubator at 25°C, and their development at this temperature followed.

In another experiment, eggs were allowed to develop at 25°C for 7 days before being placed at 4°C. After 5 months at 4°C, these eggs were returned to 25°C and their development followed. Controls were incubated in distilled water at 25°C.

RESULTS

A) Development of eggs and the hatching process.

Thirty eggs, removed from gull faeces immediately after their deposition, measured 96-126μm (mean 109μm) by 60-72μm (mean 65μm). Eggs are oval in shape, slightly asymmetrical about the long axis, and golden brown in colour. A zygote containing two pronuclei lies towards the opercular pole of the egg and measures 16-22μm in diameter (18μm in 9 of 17 observations). Boundaries between vitelline cells are very indistinct at this stage. The first few divisions of the zygote were observed to follow an asymmetrical pattern similar to that described by Pearson (1961) for Neodiplostomum (=Fibricola) intermedium. After the first 24 hours, and for the next 4-5 days at 25°C, the embryo appears as a disc of cells, at first lying close to the operculum, but becoming more centrally placed later. Boundaries between the polyhedral vitelline cells are more obvious after 5-6 days. After 7 days, the embryo is elongated, and the eyespots are visible as poorly outlined regions of dark granules. Flame cells and slight movements of the embryo may be seen at this time. Vitelline cells are now reduced in size, spherical in shape, and do not entirely fill the egg cavity. In fully developed eggs (from day 8-9 at 25°C), no structure was seen analogous with the viscous cushion at the opercular pole of Fasciola hepatica eggs. Figure 53 shows several developmental stages of the egg of D. spathaceum.

Detailed observations on the hatching process were not made. However observation led to the belief that the sequence of events is very similar to that described

by Pearson (1961) for Neodiplostomum intermedium. Pearson observed that the vitelline membrane with its enclosed miracidium is partially extruded from the egg when the operculum opens. The miracidium then ruptures the membrane and escapes. A similar process is thought to occur during the hatching of D. spathaceum eggs, but this could not be confirmed. After the operculum opens the miracidium emerges rapidly. Sometimes, however, it becomes trapped in the opercular opening and swims, trailing the shell, for a few minutes before freeing itself. In an empty shell, the vitelline membrane has come away from the wall, and is seen as a small loop suspended within the opercular opening.

B) Interpretation of experimental results

The results of each experiment were intended to indicate the rate of development of eggs, the length of their peak hatching period, and their viabilities in each situation. On six occasions, batches of eggs incubated in distilled water at 25°C, served as controls. Examination of the results from these six batches suggested a means of expressing the desired measurements.

When the percentage of viable eggs hatching in each 2-day period is plotted against time, a skewed distribution is obtained (Figure 54 - for derivation of this graph, see below). The majority of viable eggs hatch over a relatively short period, the remainder, forming the "tail" of the skewed distribution, hatching over a longer period. The length of the "tail" varied, even though batches of eggs were given identical treatments (Figure 55). Results were graphed as

cumulative percentage of viable eggs hatched by each day of observation. Points were connected by straight lines.

Similar curves were obtained for all experimental batches. In every case, over 50% of the viable eggs hatched within the period covered by the "bulge" of the skewed distribution. Graphical determination of the day by which 50% of viable eggs had hatched therefore gives a measure of the rate of development of the eggs. To estimate the length of the period of peak hatching, the number of days over which the first 75% of viable eggs hatched, was determined graphically. In most cases this corresponds closely with the period of the "bulge" of the skewed distribution.

Figure 55 gives cumulative percentage hatching curves for the six control batches. Their times for 50% hatching were 12.9, 13.8, 11.8, 14.0, 12.8, and 11.2 days. Their viabilities were 73%, 89%, 93%, 97%, 96%, and 88%, and their periods of peak hatching, 9.0, 6.8, 2.8, 4.4, 2.6, and 5.2 days respectively. If these values can be considered as samples from normally distributed populations of values, their means and standard deviations can be utilised. Thus the time of 50% hatch for the control batches is 12.8 ± 1.1 days, their viability $89 \pm 9\%$, and their period of peak hatching 5.13 ± 2.5 days. For analysis of the data these values were taken to represent the control situations. Values lying more than two standard deviations from the above means were considered to differ significantly from them. This crude statistical approach appears adequate in view of the nature of the data.

For graphical comparisons, daily values from the six control graphs. (Figure 55) have been averaged to give the combined curve in Figure 54 (used also in Figure 56). The graph of percentage of viable eggs hatching over each 2-day period, also in Figure 54, has been derived from the combined control graph.

C) Experimental results

1) Development time of eggs at different temperatures.

Figure 56 presents graphs of cumulative percentage hatch of eggs incubated in distilled water at different temperatures. Data derived from this is shown in Table 21.

Temperature differences have a more dramatic effect on the hatching times of eggs than on their viabilities or periods of peak hatching. The number of days to 50% hatch decreases exponentially with increasing temperature to a minimum at 30°C. Above 30°C, hatching time does not decrease further, and the percentage of viable eggs drops considerably. At 30°C and 20°C the percentage of viable eggs did not differ significantly from that at 25°C. Bacterial or fungal contaminants in the watch-glasses were considered responsible for the much lower viability of eggs at 10°C. Other experiments (page 161) suggested that eggs may remain viable for many months at low temperatures.

The lowest temperature for development was not exactly determined. Eggs do not develop at 4°C, and extrapolation from the curve in Figure 57 suggests that significant development would not occur below 10°C.

2) The ability of eggs to hatch in the dark.

The results obtained during two trials of this experiment are shown in Table 22. Neither the time for 50% hatch nor the viabilities of the experimental batches differed significantly from that of the combined controls. The peak hatching period could not be determined accurately from the data.

3) The ability of eggs to develop and hatch in low concentrations of oxygen, or in the presence of inhibitors of aerobic respiration.

All anaerobic jars were opened on day 15. In one of the experimental jars, 36% of the eggs had hatched, and 97% appeared viable. In the other, 95% had hatched and 98% appeared viable. In the control jar, 89% had hatched and 98% appeared viable. It was concluded that eggs can develop and hatch in low oxygen concentrations with apparently undiminished viability. It should also be pointed out that eggs in this experiment were in darkness throughout their incubation period.

The results of incubation of eggs in respiratory inhibitors are shown in Table 23. The only significant effect of respiratory inhibitors on eggs was to delay the hatching of those incubated in cyanide from day zero. Those in malonic acid, or in cyanide from day 8, did not differ from controls. Neither viabilities nor peak hatching period were significantly affected by the experimental treatments. Eggs incubated in cyanide from day 0 showed little sign of development for several days relative to controls, but subsequently developed normally.

4) Development and hatching of eggs in saline conditions.

D. spathaceum eggs are capable of normal development and hatching in all salt concentrations in which they were incubated (Table 24). One of two batches incubated in 150mM sodium chloride was delayed in hatching relative to controls. During plasmolysis experiments (see page 154) numbers of eggs were seen to hatch in 200mM sodium chloride, at which concentration some plasmolysis of the egg contents occurs.

5) The development of eggs at different pH values.

Eggs failed to develop in the acetate buffer at pH 4.0 and 5.0 during the first trial. Due to bacterial contamination of the batch at pH 9.0 in the first trial, and of that at pH 4.0 in the second, data for these are not given, although some eggs were seen to hatch in each case. Other results are shown in Table 25.

Between pH 6.0 and 8.0 inclusive, experimental batches did not differ significantly from controls for any parameter. Batches at pH 5.0 and 9.0 showed delayed hatching, and that at pH 9.0 a prolonged period of peak hatching.

6) Survival of eggs for protracted periods at low temperatures.

In incubations lasting over one month, bacterial contamination of the watch-glasses was sometimes a problem. Much quantitative data on survival of eggs at low temperatures was lost because eggs could not be clearly seen in contaminated watch-glasses.

An undetermined proportion of unembryonated eggs incubated at 4°C for six months subsequently developed and hatched on return to 25°C. Of 282 embryonated eggs (incubated at 25°C for seven days) kept at 4°C for five months, 87% hatched within 15 days of being returned to 25°C. Numbers of embryonated eggs kept at 4°C for 11 months hatched on return to 25°C.

In all experiments reported here, miracidia remained motile, and apparently unaffected by whatever medium they hatched into.

DISCUSSION

Of all experimental treatments to which eggs were subjected, incubations at different temperatures showed the most dramatic differences. Curves of hatching time against temperature constructed by Dubois (1929) for eggs of Strigea tarda (=Cotylurus cornutus) and by Rowcliffe and Ollerenshaw (1960) for Fasciola hepatica closely resemble that in Figure 57 for D. spathaceum. For both D. spathaceum and F. hepatica, development probably does not occur below 10°C, nor is development time further decreased over 30°C.

In Scottish waters at least, temperatures over 20°C are rare, and the development of eggs above this figure need not be considered. Temperatures in the River Almond at College Mill Trout Farm during 1972-1973 ranged from 0°C to 20°C (see Figure 63). Temperatures of 10°C or over were recorded between the end of April and the beginning of October, and temp-

eratures over 15°C occurred for short periods only, between June and August of each year. The greatly shortened development time above 15°C would suggest that the majority of partially embryonated eggs present in a fish farm might hatch during the short periods when temperatures rise towards 20°C. The implications are discussed further in Chapter 4.

Data given by Styczynska- Jurewicz (1965a) and by Rowcliffe and Ollerenshaw (1960) indicate that the development of eggs of F. hepatica is slowed or suspended in anaerobic conditions. The development of eggs of D. spathaceum in such conditions, and in cyanide solutions, suggests that eggs of this species are at least facultative anaerobes. Eggs in bottom sediments, where strongly reducing conditions can occur, or in stagnant ponds may thus be able to continue their development.

Styczynska-Jurewicz (1965b) has reported that eggs of F. hepatica can develop in salinities up to about 200 mM sodium chloride. D. spathaceum can also develop in saline conditions (79-92% viability in 150 mM sodium chloride). These values are above salinities recorded by Cichowlas (1961) in a part of the Baltic Sea where lymnaeids infected with D. spathaceum were found. It is likely that some fluke eggs can develop and hatch in salinities above those tolerated by their snail hosts (data in Styczynska-Jurewicz, 1965b).

Although pH values of natural waters may vary between 1.7 and 12.0 (Hutchinson, 1957 p 681), Carpenter (1928 p 68) has commented that "by common observation, animal life --- seldom occurs at all below pH 4.7 or above pH 8.5". Eggs of D. spathaceum

can develop and hatch between these values. The variability in their hatching time and viability observed experimentally are not considered of major biological significance.

The survival of D. spathaceum eggs despite prolonged incubation at low temperatures suggests that eggs can overwinter in the field. This is further discussed in Chapter 4.

Specific stimuli are often considered essential for hatching of operculate trematode eggs (see Erasmus, 1972 p 49). Eggs of D. spathaceum, however, appear capable of hatching without apparent stimuli under any conditions in which they can develop.

Light has been considered a neccessary stimulus for the hatching of many eggs (Rowcliffe and Ollerenshaw, 1960, and Rowan, 1956 for F. hepatica). Amongst the strigeoids, eggs of Alaria spp. require light for normal hatching (Pearson, 1956) whereas those of Fibricola cratera do not (Hoffman, 1955). Hilliard (1960) observed that eggs of some species of the pseudophyllidean genus Diphyllobothrium require light as a hatching stimulus, whereas others do not. Similar variability is not unexpected therefore among the trematodes.

Rowan (1957) and Wilson (1968) working on F. hepatica, and Pearson (1961) on Neodiplostomum (=Fibricola) intermedium, considered that hatching is preceded by an inflow of water into the egg. This increases internal pressure and helps to rupture the operculum. Contents of embryonated D. spathaceum eggs suffered plasmolysis in 200 mM sodium chloride, but were capable of hatching in this solution. Simple

dilution and swelling of egg contents by an inflow of water is not a plausible explanation of hatching under such conditions. Eggs of D. spathaceum and N. intermedium (see Pearson, 1961) lack the viscous cushion, common to many trematodes, which expands by hydration to increase internal pressure during hatching. The nature of the cushion material in F. hepatica is such that it can expand in 170 mM sodium chloride, at which salinity the remaining egg contents lose water osmotically (Wilson, 1968). The hatching mechanism in strigeoids must differ somewhat from that in F. hepatica. Pearson (1961) suggested that material between the shell and vitelline membrane of embryonated N. intermedium eggs may play a part in hatching. If this should prove similar in nature to viscous cushion material, the paradox of eggs hatching in isotonic or hypertonic solutions may be resolved.

During their development, trematode eggs may be ingested by snails. Many species have evolved to exploit this possibility, only hatching after being eaten by a snail (Erasmus, 1972 p 46). Fully developed D. spathaceum eggs were occasionally seen to be ingested by Lymnaea peregra, but appeared later in the faeces, unhatched but still viable.

SUMMARY

1) The development and hatching of eggs of a representative strigeoid (Diplostomum spathaceum) were briefly described.

2) Experimental studies on D. spathaceum eggs revealed that they are capable of normal development and

hatching under all conditions of pH, salinity, and oxygen concentration in which snail hosts are likely to occur. Their development rate appears negligible below 10°C, but increases exponentially with temperature to a maximum at about 30°C. The eggs are capable of hatching in the dark, and of surviving long periods at low temperatures.

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EXPLANATORY NOTE FOR TABLES 21-25

Some column headings in these tables could not be made both brief and entirely self-explanatory.

The "Day of 50% Hatch" column gives the number of days from the start of an incubation until 50% of the viable eggs had hatched. This gives a measure of the rate of development of the eggs.

The "Days for 75% Hatch" column gives the number of days over which the first 75% of viable eggs hatched. This gives a measure of the length of the hatching period.

The same control values were used in each experiment, expressed as the mean of values from several control batches and the standard deviation of these values. In each column "$\pm$ S.D.'s from Control", the figure quoted is the number of standard deviations (of the control batches) by which the experimental value differs from the control mean.

A full explanation may be found in the text, on page 157.

TABLE 21: Times for 50% hatch, viabilities, and periods of peak hatching of *Diplostomum spathaceum* eggs incubated at different temperatures

Temperature	Day of 50% Hatch	± S.D.'s from Control	% Viable	± S.D.'s from Control	Days for 75% Hatch	± S.D.'s from Control
40°C	8.6	3.8	23	7.3	4.4	<1
30°C	8.0	4.3	90	<1	5.0	<1
(2 batches)	8.8	3.6	95	<1	5.8	<1
25°C (combined value for the 6 control batches)	12.8 ± 1.1	-	89 ± 9	-	5.13 ± 2.5	-
20°C	19.4	6.0	77	<2	7.6	<1
(2 batches)	19.7	6.3	98	<2	7.4	<1
15°C	40.4	25	69	2.2	20.4	16
10°C	142	117	38	5.7	50	18

TABLE 22: Times for 50% hatch, and viabilities of batches of D. spathaceum eggs maintained in the dark

Batch	Day of 50% Hatch	± S.D.'s from Controls	% Viabilities	± S.D.'s from Controls
Trial 1	11.2	<2	89 - 92	<1
Trial 2	13.4	<1	96 - 98	<1
Combined Controls	12.8 ± 1.1	-	89 ± 9	-

TABLE 23: Times for 50% hatch, viabilities, and periods of peak hatching of D. spathaceum eggs incubated in respiratory inhibitors

Treatment	Day of 50% Hatch	± S.D.'s from Controls	% Viability	± S.D.'s from Controls	Days for 75% Hatch	± S.D.'s from Controls
Cyanide from Day 0						
Trial 1	15.8	2.7	94	<1	4.6	<1
Trial 2	20.0	6.6	93	<1	9	<2
Trial 3	17.8	4.5	95	<1	4.5	<1
Cyanide from Day 8	14.4	<2	96	<1	7.2	<1
Malonic Acid	12.4	<1	96	<1	2.8	<1
Combined Controls	12.8 ± 1.1	-	89 ± 9	-	5.13 ± 2.5	-

TABLE 24: Times for 50% hatch, viabilities, and pericds of peak hatching of D. spathaceum eggs incubated in different salinities

Treatment	Day of 50% Hatch	± S.D.'s from Controls	% Viability	± S.D.'s from Controls	Days for 75% Hatch	± S.D.'s from Controls
10 mM Saline	14.4	<2	98	1	5.4	<1
50 mM	15.5	<2	92	<1	8.8	<2
150 mM Trial 1	18.6	5.3	79	<2	7.5	<1
150 mM Trial 2	14.2	<2	92	<1	4.8	<1
Combined Controls	12.8 ± 1.1	-	89 ± 9	-	5.1 ± 2.5	-

TABLE 25: Times for 50% hatch, viabilities, and periods of peak hatching for *D. spathaceum* eggs incubated at different pH values

pH	Day of 50% Hatch	± S.D.'s from Controls	% Viability	± S.D.'s from Controls	Days for 75% Hatch	± S.D.'s from Controls
5, Trial 2	16.7	3.5	93	<1	4.6	<1
6, Trial 1	14.2	<2	90	<1	5.0	<1
6, Trial 2	12.8	<1	96	<1	3.0	<1
7, Trial 1	12.3	<1	92	<1	3.4	<1
7, Trial 2	12.5	<1	95	<1	4.0	<1
8, Trial 1	11.5	<2	93	<1	2.8	<1
8, Trial 2	14.8	<2	94	<1	7.8	<2
9, Trial 1	17.4	4.2	73	<2	14.2	3.5
Combined Controls	12.8 ± 1.1	-	89 ± 9	-	5.13 ± 2.5	-

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Figure 53 Diagrams of some developmental stages of the egg of Diplostomum spathaceum.

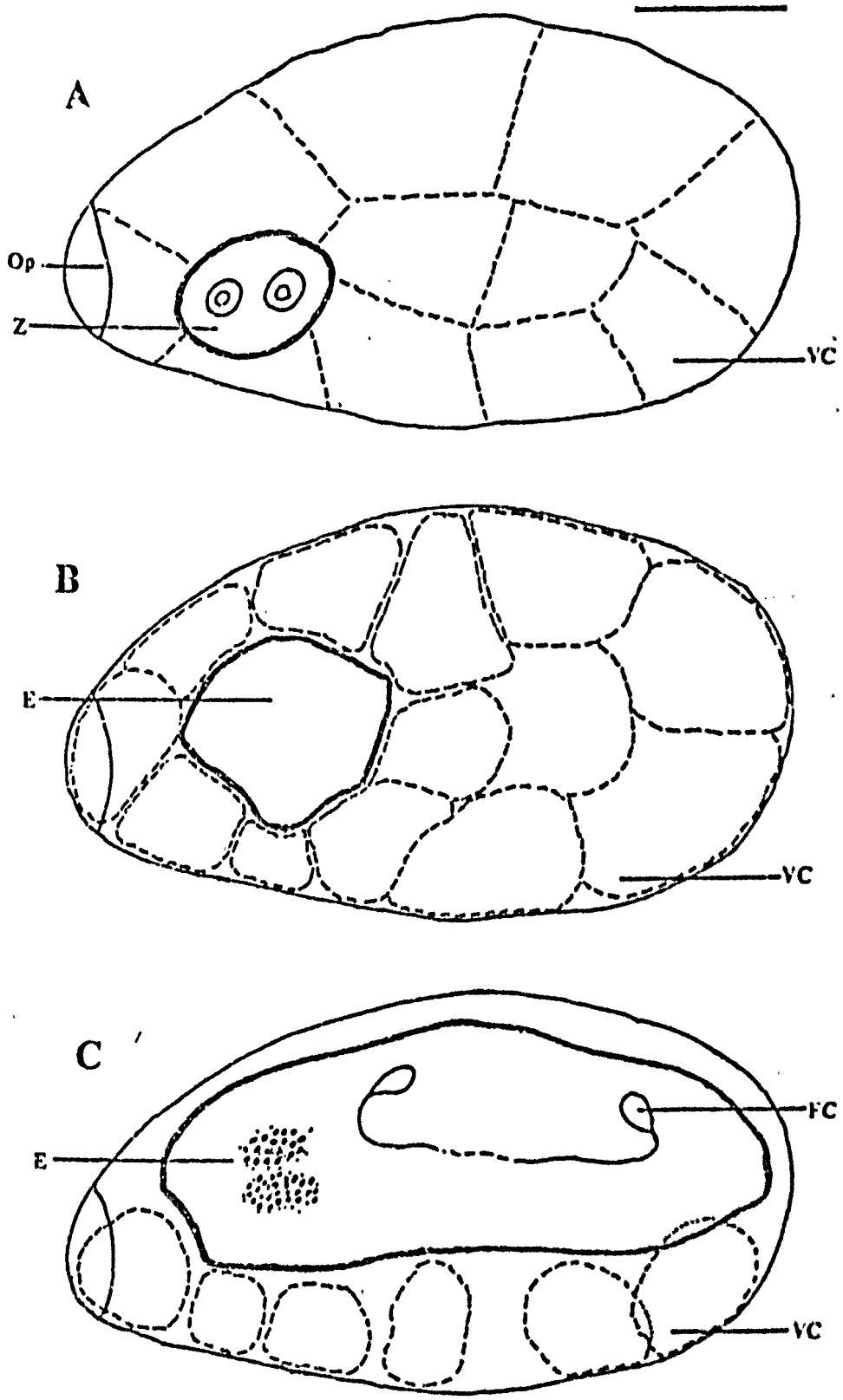
A) On day zero. The zygote contains two pronuclei.

B) On day four. The embryo lies towards the opercular pole of the egg.

C) On day eight. The embryo is well developed and contains eyespots and flame cells. Note the decrease in the size of the vitelline cells.

The bar represents approximately 20μm.

key to abbreviations on page xi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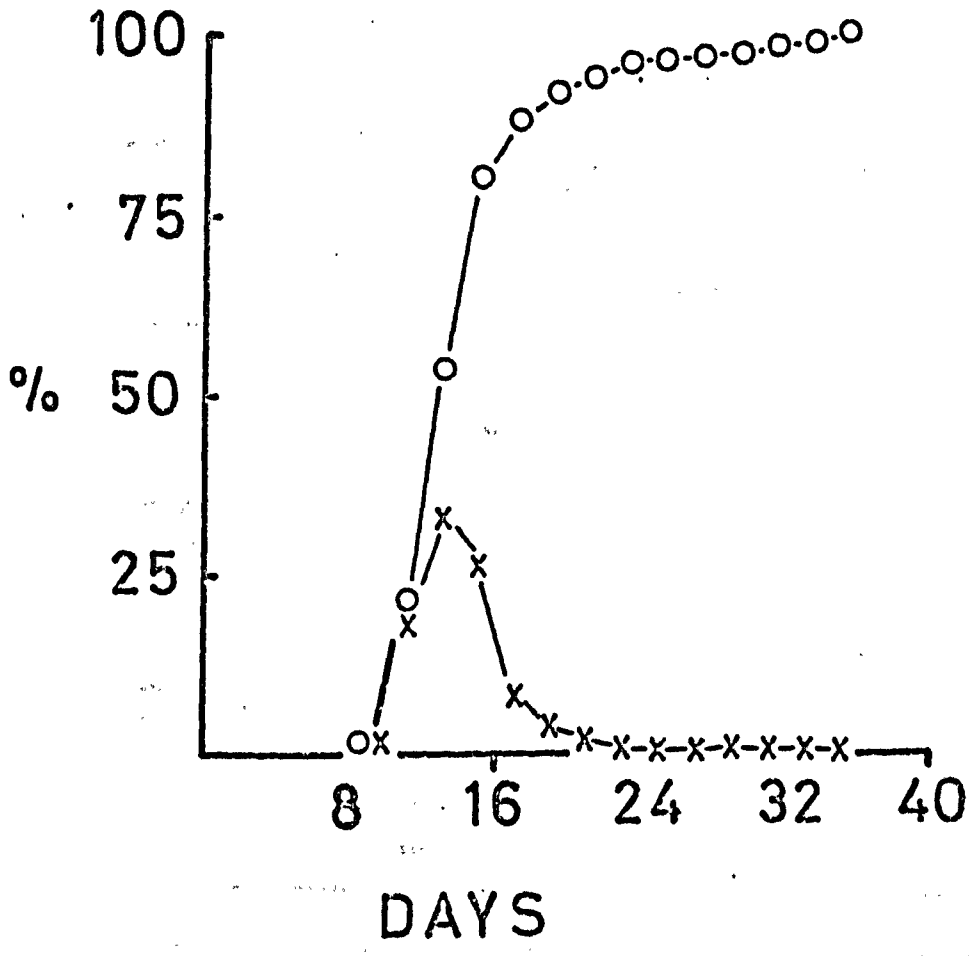

Figure 54 Upper curve; combined control curve obtained by averaging daily values for cumulative percentage hatch of the six control batches of Diplostomum spathaceum eggs incubated in distilled water at 25°C.
Lower curve; The percentage of viable D. spathaceum eggs hatching in each 2-day period. Derived from the upper curve.

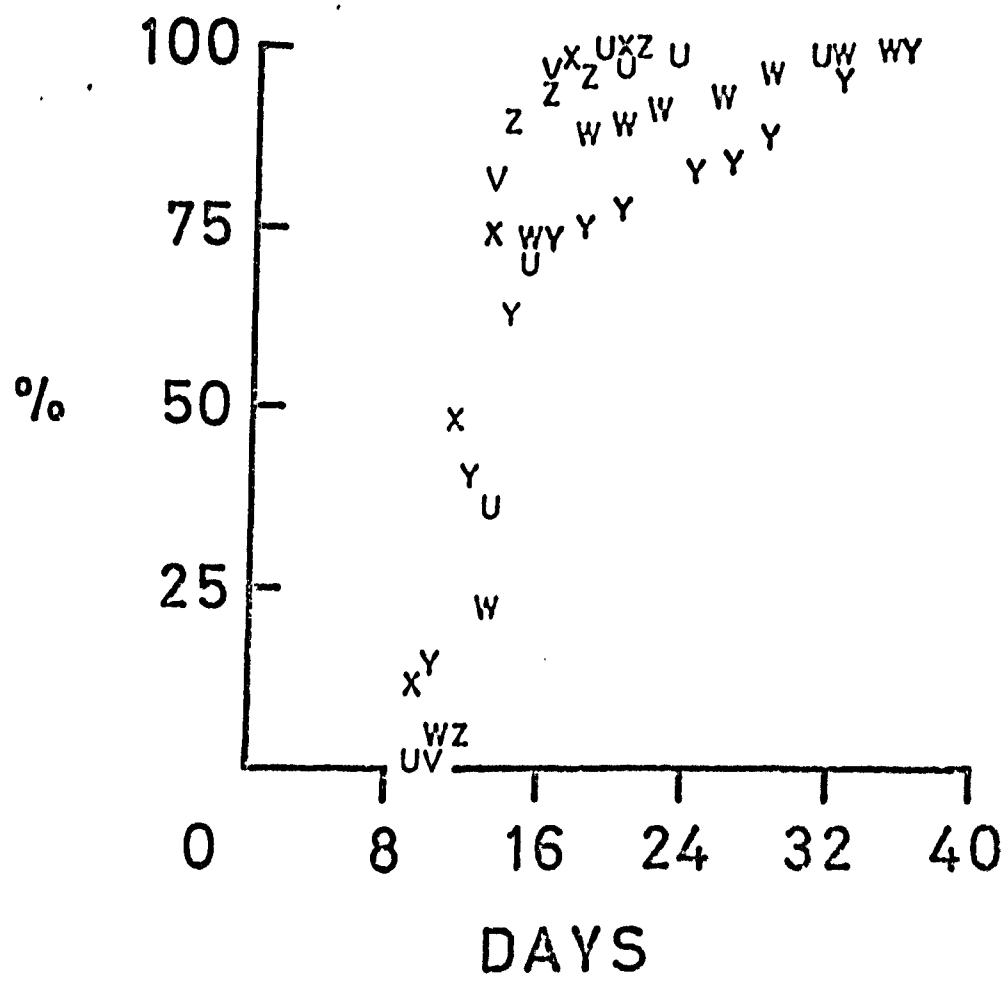

Figure 55 Cumulative percentage hatch of eggs in each control batch incubated in distilled water at 25°C. Each letter represents values for a different batch.

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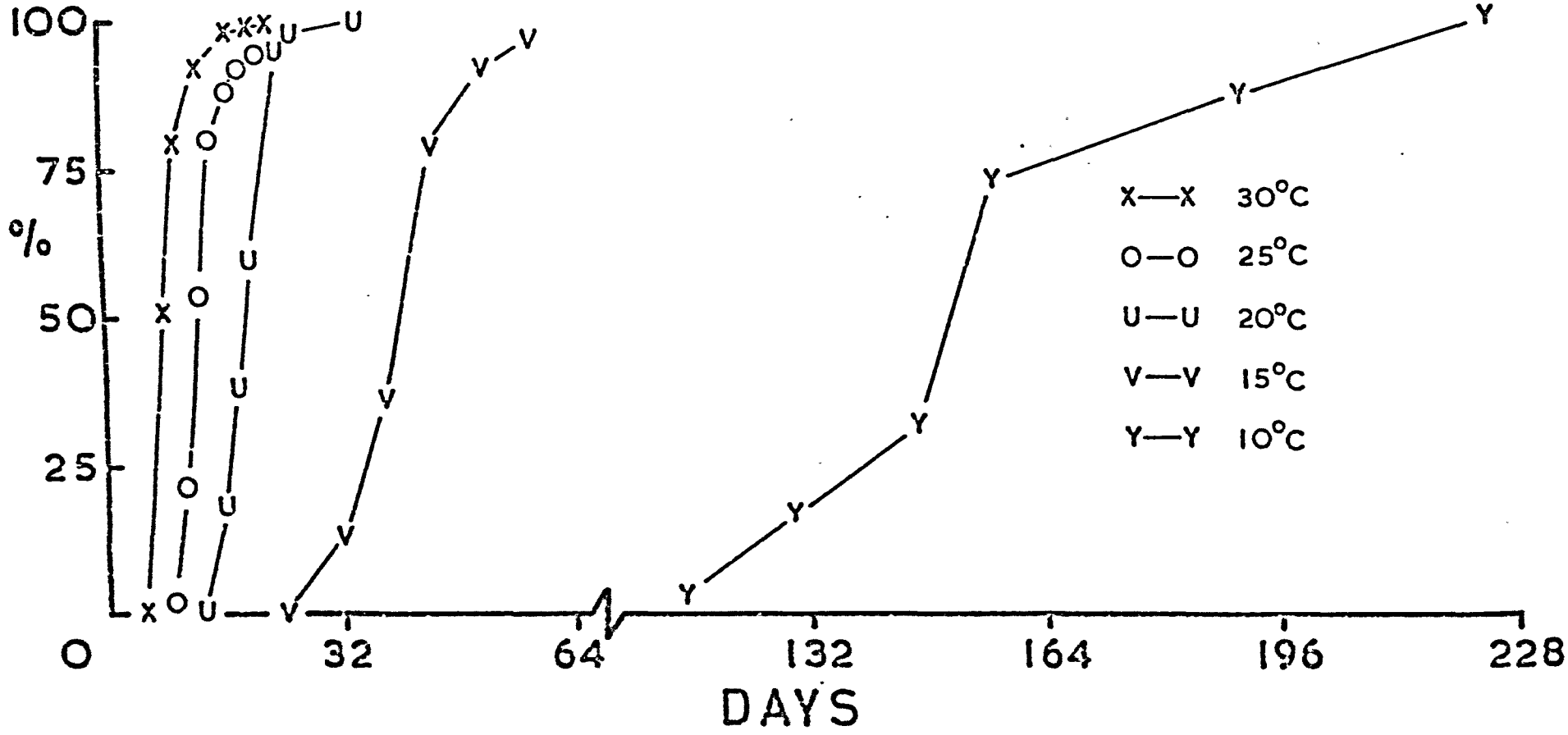
Figure 56 Cumulative percentage hatch of Diplostomum spathaceum eggs incubated in distilled water at different temperatures. The combined control curve is used for the batches incubated at 25°C. Only one curve is drawn for each of the two trials at 30°C and at 20°C.


100
75
%
50
25
0
32
64
132
164
196
228
DAYS
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O—O 25°C
U—U 20°C
V—V 15°C
Y—Y 10°C

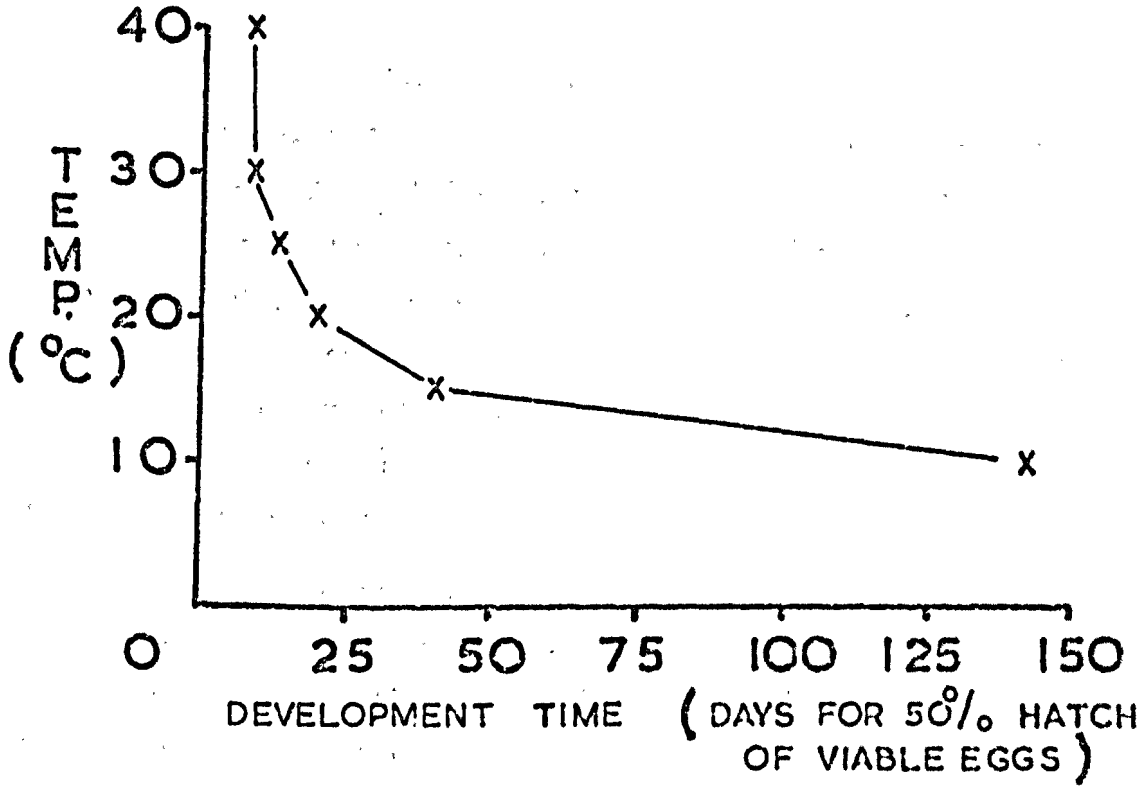


Figure 57 Graph of the development time (number of days for 50% hatch of viable eggs) of Diplostomum spathaceum eggs at different temperatures.

CHAPTER 4

EPIDEMIOLOGY OF STRIGEOID INFECTIONS OF SNAILS AND FISH

INTRODUCTION

At College Mill Trout Farm, a supply lade or channel from the River Almond feeds, in parallel, a number of indoor concrete tanks and 16 outdoor earth-banked rearing ponds, the latter 25 by 5m by 1.5m deep. The owner buys rainbow trout eggs from Europe (in spring) and from Tasmania (in autumn). Fry hatching from these are maintained for several months in the indoor tanks before being transferred to the outside rearing ponds.

Strigeoid metacercariae were found in fish both in the indoor fry tanks and in the outside rearing ponds. Snails occurred in the supply lade and in the rearing ponds, but not in the fry tanks. Possible sources of cercariae were therefore snails in the water supply and those in the rearing ponds.

A marked summer increase in infection levels of fish with metacercariae was observed by Campbell (pers. comm.), coinciding with the latter part of the snail breeding season, and at a time when higher water temperatures should lead also to hatching of trematode eggs (see Chapter 3). Snails at College Mill Trout Farm were sampled from May 1972 until December 1973 in an attempt to analyse their population structure, and understand the epidemiology of the trematodes. Corroborative data obtained by sampling fish from the farm has been supplied by Mr. A.D. Campbell of the Department of Agriculture and Fisheries for Scotland, to whom the author is greatly indebted.

MATERIALS AND METHODS

A) Collection and examination of snails

Normally, each rearing pond at College Mill Trout Farm is drained once or twice a year and treated with copper sulphate to kill snails. However, one pond, pond 13, had not been treated since 1970, and contained large numbers of snails when sampling started in 1972. Samples were therefore taken from this pond from May 1972 until October 1972, when it was finally drained. Subsequent samples were taken from pond 7 which had been treated and refilled during August 1972. Snails recolonised pond 7 during the autumn and winter of 1972 and large numbers were found early in 1973 (Campbell (pers. comm.) estimated there to be 1800 snails in pond 7 on 15 May 1973).

Samples were taken by drawing a hand net several times along the substrate of the pond from its deepest part up to the marginal vegetation. The net retained all objects above about 0.5mm diameter. Its contents were brought back to the laboratory and placed in a white polythene tank, covered with water, and kept at about 20°C. Frequent examination under a lamp revealed snails on the sides of the tank and on the surface of the pond debris. By this method it was possible to find even very small snails which might have been difficult to separate from the substrate by other means. Generally, all live snails were seen and removed from the tank within 4 or 5 days.

Examination of snails was carried out as soon as possible after their collection. The maximum shell length of each snail was measured to the nearest 0.1 mm

using Vernier calipers. The majority were then dissected in Lymnaea saline (Carriker, 1946). Any trematodes found (sporocysts, rediae, and metacercariae) were identified as far as possible, and their presence recorded. Infections in snails which had been shedding cercariae, or which yielded apparently fully developed cercariae on dissection, were classed as "mature".

While taking each sample, the pond was examined for the presence of snail eggs, and the relative abundance of these assessed. Water temperatures were recorded in the early afternoon each day from the supply lade at College Mill Trout Farm.

B) Examination of fish

Samples of fish were removed at weekly or fortnightly intervals from fry tanks and selected rearing ponds for examination by Mr. A.D. Campbell. Ten fish were generally taken on each occasion. The numbers of metacercariae in their lenses and humours were recorded separately. The numbers of "small forms", considered to represent newly penetrated cercariae, were also noted.

C) Experimental work

An experiment was set up to assess the development time of Diplostomum spathaceum within snails at different temperatures. About 100 Lymnaea peregra were exposed to infection by miracidia of D. spathaceum at 20°C. Twenty-four hours later, groups of snails were removed to tanks at 10°C, 15°C, and 20°C. Snails were removed at intervals and dissected to determine the development of their infections.

RESULTS

A) Life-cycles of the snails.

The only snail occurring commonly at College Mill Trout Farm was Lymnaea peregra. A very small number of Lymnaea truncatula were also found during sampling, but none was infected. Shell lengths of L. peregra sampled from ponds 7 and 13 are shown in Figure 58. The data has been gathered into size classes of 0.5mm for presentation. Space did not permit the inclusion in the figure of the sample from pond 7 on 31 July 1973. The sample from this pond taken on 9 July 1973 fell clearly into two size groups, larger snails belonging to the overwintering generation, and smaller, recently hatched snails (see Figure 58). So many small snails were present that a further sub-sampling of these was required, about 1/16 of the total number being measured. The proportion of large snails in the 9 July 1973 sample is thus exaggerated some 16-fold in Figure 58.

There are several differences between the life-cycles of Lymnaea peregra in pond 13 during 1972 and those in pond 7 during 1973. In pond 7, snails of the generation overwintering since 1972 increased in size during the spring, and died out during July. Their egg masses were found between mid-May and the end of July, with a peak of abundance in June. Young snails were found for the first time, and in enormous numbers, on July 9th. Their average shell length increased gradually over the next few months to give a size distribution by December 1973 rather similar to that of the previous generation in March 1973. Thus in pond 7, Lymnaea peregra appeared to undergo a simple annual life-cycle, an overwintering generation of

snails breeding in mid-summer and being replaced by snails of the next generation which will themselves overwinter.

The situation in pond 13 during 1972 is less easily interpreted. Egg masses were present in early April (no snail sample taken) and most abundant in May. Fewer were seen in early July, but a distinct increase was noted in August.

When the 9 May and 30 May samples from pond 13 are compared, the latter has a much lower proportion of snails over 10 mm and a higher proportion under 6 mm (Figure 58). In Figure 59 are compared the numbers and sizes of snails (grouped in 1 mm size classes) infected with Cotylurus cornutus metacercariae with those uninfected in the two samples. Their distribution cannot be fitted to a normal distribution for simple statistical analysis. Examination of the figure, however, reveals striking differences between the samples. In the 9 May sample, the distributions of infected and uninfected snails are similar and the majority are infected. In the 30 May sample, a high proportion is uninfected and their average size is substantially below that of the infected. The majority of uninfected snails in the 30 May sample lie below the lower end of the size distribution of the 9 May sample. Since fresh infection of snails as early as May is not likely (see below), infected snails must have acquired their metacercariae prior to overwintering. Metacercariae probably do not contribute to mortality in their hosts. The data is interpreted as demonstrating that, by the 30 May sample, larger snails of the overwintering generation had laid their eggs and were dying off. The majority

of uninfected individuals were small and represent spat hatching from eggs laid during April and May.

Although the 6 July sample was small, it appears to indicate rapid growth of the spring generation and survival of few overwintering snails by this time. Snails of the spring generation are presumed to be responsible for egg production in August, leading to the appearance of a late summer generation. It could not be determined whether snails of the spring generation died out completely, being replaced by the late summer generation, or whether the population which would have subsequently overwintered included individuals of each generation.

B) Infections in the snails.

Echinostome and monostome cercariae, xiphidiocercariae and furcocercariae were recorded from L. peregra from College Mill Trout Farm. Amongst the furcocercariae, schistosomatids were occasionally observed, the remainder being strigeoids. Metacercariae of echinostomes, monostomes, and of Cotylurus cornutus were commonly found during dissection of snails. Table 26 and Figure 60 record infection levels of sporocysts and rediae and of C. cornutus metacercariae from L. peregra over the period of study. Where samples were very large, not all snails were dissected. Those measured without being dissected tended to be smaller than average, since these were often the last to be picked from the sediment sample. Possible bias due to this should be borne in mind when data in Table 26 is analysed.

The proportion of snails containing mature infections of any sort was very low. Of 3071 L. peregra dissected

during 1972-1973, 129 (approximately 4%) were so infected, and of these, only 32 (1%) contained strigeoids. The samples in which strigeoid infections occurred are shown in Table 27.

Certain trends are apparent from Table 26. The proportion of infected snails drops considerably after the breeding season, as older snails die off, and young snails enter the population (compare 9 May and 30 May 1972 samples, 6 July with 15 August 1972, and 9 July over-wintering generation sample with the 31 July 1973 summer generation sample). Thereafter infection levels in the new generation of snails gradually build up. Towards the end of a breeding period, snails of the older generation show a substantial increase in infection levels (e.g. 6 July 1972 sample, and 9 July 1973 older generation).

On 31 July 1973, only four snails of the older generation were taken in the routine sample from pond 7. The pond was then drained, to allow removal of fish, and refilled. While empty, numbers of snails were seen which, by their size, must have belonged to the older generation. Thirty-one of these, measuring 11.6-18.8 mm were brought back to the laboratory. Sixteen were shedding xiphidiocercariae, one shed schistosomatids, two shed Apatemon gracilis, one shed Diplostomum phoxini, three shed Diplostomum sp., and one shed a Diplostomum (Tylodelphys) sp. Thus 24 of 31 contained mature infections, and a further four contained immature rediae.

C) Infections in the fish.

Sampling of fish during 1972 revealed much higher

infection levels in the outside rearing ponds than in the fry tanks. Of 20 fish taken from a fry tank on 20 July 1972, only two contained metacercariae. By contrast, all 10 fish removed from pond 12 on the same day were infected, with a mean of 118 strigeoid metacercariae in the eyes of each fish. This confirmed the main source of infection to be the snails within the rearing ponds.

Large numbers of metacercariae built up during the summer months in fish in rearing ponds which had not been treated recently with copper sulphate. The identification of these has been discussed in Chapter 2. Figure 61 shows the mean numbers of metacercariae in eyes from samples of 10 fish removed at intervals from pond 12 during 1972. The heavy infections in the lenses by mid-July caused severe damage to the lens and blindness. Smaller numbers subsequently recorded from the lenses are thought to reflect death of parasites in the now uninhabitable lens. (Death of D. spathaceum metacercariae following lens rupture has been observed by Sweeting, 1971 p 128). It is noteworthy that metacercariae in the vitreous humours of pond 12 fish accumulated steadily into September. Assuming that metacercariae in the vitreous humours were not forms released by rupture of the lens, cercarial production appears to have occurred throughout this period.

Figure 62 shows infection levels in fish from pond 5 during 1971 and may reflect a more typical situation. Again numbers of metacercariae built up during the second half of June. The intensity of infection, however, was lower than in pond 12 in 1972, and the lack of a dramatic decrease in lens metacercariae after July suggests a

lower incidence of lens rupture. The numbers of recently penetrated metacercariae showed a small peak in July and a larger one in late August, indicating maxima of cercarial production at these times.

Previously uninfected rainbow trout placed in pond 7 in mid-June 1973 were heavily infected with strigeoids by 9 July. By 31 July, five fish each contained an average of 73 metacercariae of Apatemon gracilis (mainly within the pericardium). Fish from the same source added to pond 7 on 8 August 1973 each contained few (<10) metacercariae when removed on 16 September. Evidence for a late summer peak of infection is therefore lacking in this case.

D) Experimental work on snail infections.

Those Lymnaea peregra exposed to miracidia of D. spathaceum in the laboratory, then maintained at 15°C and 20°C, ultimately yielded cercariae. At 20°C the prepatent period of D. spathaceum is 4 weeks, and at 15°C, between 8 and 9 weeks. After 12 weeks, snails maintained at 10°C contained only mother sporocysts which lacked well developed daughter sporocysts.

Snails shedding cercariae after 3 months at 15°C were transferred to a tank at 12°C. Cercariae continued to be shed at this temperature, but were not after a further transfer of the snails to 10°C. In these experiments, therefore, the minimum temperature for shedding appears to lie between 10°C and 12°C.

DISCUSSION

A) Life-cycles of the snails

Hunter (1961b) concluded that an annual life-cycle is general among Scottish freshwater snails (an exception is Lymnaea stagnalis, see Berrie, 1965), with a single period of egg laying in the summer followed by death of the older snails. In the case of L. peregra, however, Hunter found populations in which two generations occur in a single year, one appearing in spring, and completely replaced by a generation of autumn bred snails. Populations in which a spring generation was only partly replaced by autumn bred snails were also reported. Hunter considered that "the actual pattern of life-cycle, though varying within the species, seems to remain constant within each population", a statement which the present data seems to contradict (Figure 58). Furthermore, "there is a minimum size (and a minimum mean size of the population) for the onset of breeding". For a population of L. peregra with a simple annual life-cycle in Loch Lomond, Hunter gives a mean size of 8.5 mm at the time of maximum reproductive activity. This figure is surpassed by the 9 May 1972 sample from pond 13 (mean 10.4 mm) and the 14 June 1973 sample from pond 7 (mean 11.4 mm), on which dates egg production was judged at its peak.

From this background, a model for the life-cycle of L. peregra at College Mill Trout Farm may be constructed. Prior to the start of sampling in May 1972, conditions at the trout farm must have been such as to permit the growth of snails beyond the minimum breeding size. Egg laying could therefore start with the increase in water temperature in early spring (April). Sufficient

time was therefore available for some or all snails of the spring generation to mature and breed before falling water temperatures in autumn inhibited this. (Hunter, 1961b has also observed that, where more than one generation occurs in a year, "overwintered adults are larger than the spring born generation at their respective periods of reproduction". This appears to be borne out by the pond 13 data in Figure 58.)

The mean size of the population overwintering into 1973 (pond 7 data) was far below the minimum for maturation. Since little or no growth was apparent between October 1972 and February 1973 (if snails in ponds 7 and 13 are considered as representatives of the same population), overwintered snails spent the spring and early summer growing and maturing to breed in mid-summer (June). The resultant spat therefore had an insufficient period for maturation before the winter.

In this model, the numbers of generations produced in a given year will depend on the mean shell length of the population overwintering from the previous year. Overwinter growth of L. peregra depends to an extent on the severity of the winter (Hunter, 1961a). Unfortunately, water temperatures were not recorded during the winter at College Mill Trout Farm. There was evidence from another source however (Calow, pers. comm.), that spring breeding of L. peregra at other localities in Scotland was exceptionally late in 1973. General climatic conditions in 1972 and 1973 may thus account for the differing life-cycles of L. peregra during these two years.

It should be borne in mind that the draining and treatment of pond 7 in 1972 may have influenced in some

way the growth of snails subsequently recolonising it. This could have led to the appearance of a population of small mean shell length over the winter months. The spring growth period required by these snails would then result in late breeding.

B) Epidemiology of the strigeoid infections.

Although a number of authors have observed a bimodal annual distribution of larval trematode infections in snails (Miller and Northup, 1926; Rees, 1932; Rankin, 1939, and Probert, 1966b), few have attempted to correlate snail life-cycles with parasite incidence. Sewell (1922 p 13) was the first to consider that natural mortality of older snails at specific seasons may contribute to fluctuations in infection levels. Recently, as evidenced by the work of Ollerenshaw (1959) on the epidemiology of Fasciola hepatica in Wales, the significance of snail life-cycles has become more appreciated. The present data permit some discussion on aspects of the interrelationships of snail and trematode life-cycles, and how these result in infection of fish.

1) Period of infection of snails.

Very few snails were found on dissection to contain "old" or "spent" redial or sporocyst infections. For this reason, and because of the short life-span of L. peregra at College Mill Trout Farm, it is assumed that a snail there does not outlive its trematode infections.

As shown in Chapter 3, eggs of at least one strigeoid, D. spathaceum, are capable of surviving long periods at winter temperatures, and of hatching when the water

temperature rises above about 10°C. Daytime water temperatures were above 10°C between mid-May and mid-September in 1972 and 1973 (Figure 63). Those trematode eggs present in the rearing ponds in May should include embryonated eggs from the previous year, as well as unembryonated eggs from faeces deposited over the winter. A steady rate of hatching of miracidia from May onwards could therefore be expected from overwintered eggs.

Nesting and brood raising by birds in early summer is associated with an increase in their parasite fauna (Dogiel, 1962). An increase in egg deposition in bird faeces is likely at this time (e.g. from black-headed gulls nesting close to the trout farm), leading to a peak of egg hatching probably in July.

Water temperature will influence the pattern of egg hatching, and hence infection rate of snails. Short periods of very warm weather would be likely to bring many eggs to the point of hatching. The 9% infection (with rediae and sporocysts) of pond 13 snails in August 1972 was well above that for pond 7 snails in August 1973 (Table 26). The latter, however, had a higher infection level by October. Water temperatures in July 1972 reached 20°C for several days, whereas in July 1973, they fluctuated between 14°C and 17°C. Possibly the short, very warm spell in 1972 brought about a peak of egg hatching in July which did not occur until later (August-September) during the more equable summer of 1973.

2) Survival of infected snails.

There is no general agreement in the literature (Wright, 1971 p 121) as to the long term effects on snails

of redial or sporocyst infections. Some records (Vernberg and Vernberg, 1963; Barbosa, 1962) indicate that snails may be killed by their infections, or at least made more susceptible to environmental fluctuations. However, experiments by McClelland and Bourns (1969) demonstrated that Lymnaea stagnalis infected with Trichobilharzia ocellata live longer and grow larger than controls.

At College Mill Trout Farm, the great increase in infection levels in old snails late in the breeding season cannot be entirely due to a large miracidial hatch at this time. In the 31 July supplementary sample from pond 7 in 1973 (see page 183), 24 of 31 snails contained mature infections. At the recorded temperatures (Figure 63), infections must have been acquired at least 2 months earlier to have matured by 31 July. There had been no corresponding increase in infection levels during May 1973, and young snails, already abundant on July 9th, showed a low incidence of infection. A differential survival of infected snails beyond the normal die-off time for older snails explains these observations most readily.

Snails of the supplementary sample were very large (page 183). Gigantism of infected snails as reported by various authors (Wright, 1971 p 119) may reflect in part their greater longevity.

3) Patterns of cercarial production.

At 15°C and 20°C the development time for D. spathaceum in L. peregra is 8-9 weeks and 4 weeks respectively. During the warmest part of the summer (at

1973 temperatures) an infection should take about 6-8 weeks to mature. The prepatent period would be longer both earlier in the summer, and towards the autumn.

Strigeoid metacercariae began to build up in fish and in snails by mid-June during 1972 and 1973 (Table 26 and Figures 60, 61, and 62). This was shortly after the water temperature had risen above the 10-12°C minimum for cercarial emergence. Snails producing these cercariae must have acquired their infections the previous year, as insufficient time had elapsed for infections of that year to mature. The greater longevity of infected snails extends their period of shedding well into the summer.

At least during 1971 and 1972, there was evidence of a second build up of fish infection levels during August (Figures 61 and 62). Perhaps because of the warm July and the early breeding period in 1972, young snails of that year were already shedding cercariae by mid-August, accounting for the increase in fish infection then. In pond 7 during the more equable summer of 1973, young snails appeared in early July. With the exception of a single individual taken on 31 July, none of these yielded cercariae until between mid-August and mid-September. A second peak of fish infection did not appear to occur at this time, perhaps because of falling temperatures and the low incidence of strigeoids in the snail population.

The appearance of a late summer peak of fish infection is likely to be influenced, therefore, by the period of breeding of the snails, and by the water temperatures in August and September.

It need not cause surprise that a small proportion of infected snails can lead to massive metacercarial infections in fish and (in the case of C. cornutus) in other snails. Individual snails from College Mill Trout Farm were found to shed between 500 and 20,000 furcocercariae per day in the laboratory (Campbell, pers. comm.). It should also be noted that infected snails tend to be larger, and can therefore sustain greater yields of cercariae (Cort et al., 1957).

SUMMARY

1) The life-cycle of Lymnaea peregra at College Mill Trout Farm was followed during 1972 and 1973. During 1972 there were peaks of egg laying by L. peregra in May and in August, with the May peak the larger. Young snails appeared in the population after each peak. During 1973 a single period of egg laying occurred, with a peak in June. The differing life-cycles in these two years may have been due to climatic differences influencing the mean shell length of the populations, or to the draining and treatment in 1972 of the pond sampled in 1973. The former interpretation is preferred.

2) Each peak of egg laying was followed by the death of the older snails. At the end of a breeding period, few large, old snails could be found, and these were generally infected with rediae or sporocysts. Infected snails appeared to live longer and grow larger than uninfected. The first increase in infection levels of strigeoid

metacercariae in fish occurred in June in 1972 and 1973. Cercariae emerging from old snails infected the previous year were responsible for this. When the snails reproduced sufficiently early in the year, trematode infections in the young snails could mature before the water temperature fell in the autumn. Cercariae from these snails may have been responsible for a second increase in fish infection levels late in the summer.

TABLE 26: Numbers and percentages of Lymnaea peregra, collected at College Mill Trout Farm during 1972-1973, infected with larval trematodes

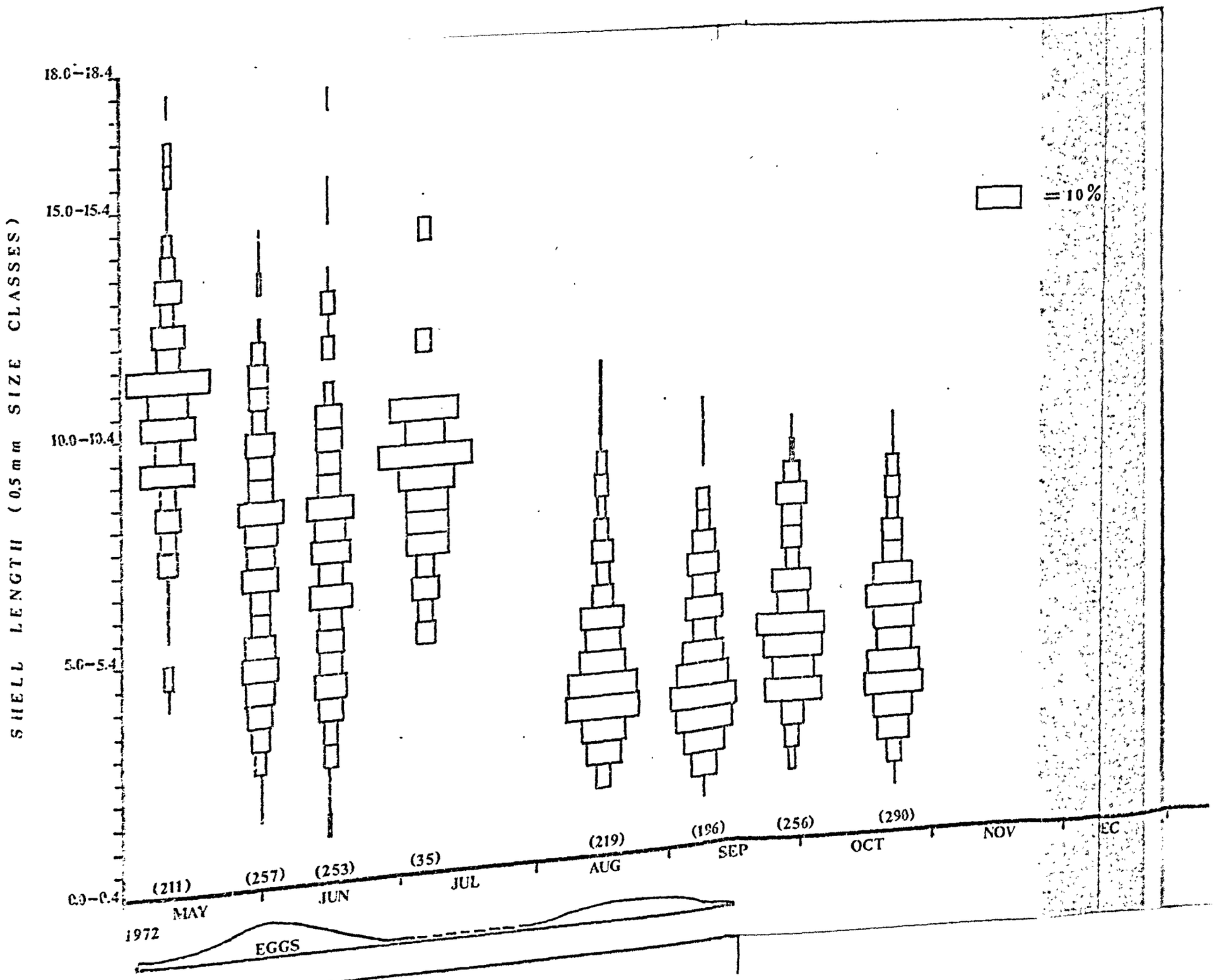
		No. Examined	Total in Sample	Infected Rediae/Sporocysts		Shedding Anything		Shedding Strigeoids		C. cornutus Metacercariae	
Pond 13	9 May 1972	211	211	(46)	21.8%	(30)	14.2%	(14)	6.6%	(151)	72%
	30 May 1972	257	257	(18)	7.0%	(11)	4.3%	(2)	0.8%	(122)	47.5%
	14 June 1972	210	253	(25)	11.9%	(11)	5.2%	(6)	2.9%	(138)	66%
	6 July 1972	30	35	(8)	26.6%	(6)	20%	0		(28)	93%
	15 Aug. 1972	89	219	(8)	9%	(2)	2.2%	0		(20)	22%
	8 Sept. 1972	94	196	(9)	9.4%	(1)	1%	(1)	1%	(37)	39.4%
	28 Sept. 1972	170	256	(12)	7.1%	(5)	3.0%	(1)	0.6%	(91)	53.5%
	21 Oct. 1972	171	290	(17)	9.5%	(4)	3%	(3)	2.3%	(106)	62%
Pond 7	15 Feb. 1973	241	308	(5)	2.1%	0		0		(17)	7%
	15 March 1973	169	205	(5)	3%	0		0		(16)	9.5%
	19 April 1973	36	36	(1)	2.8%	0		0		(1)	2.8%
	18 May 1973	179	187	(11)	6.1%	(5)	2.8%	0		(10)	5.6%
	14 June 1973	101	107	(6)	6%	(6)	6%	0		(48)	47.5%
Old Generation	9 July 1973	61	61	(24)	39%	(5)	8.2%	(1)	1.66%	(39)	64%
Old Generation	31 July 1973	3	4	(3)		(3)		0		(2)	
New Generation	9 July 1973	81	150	(2)	2.5%	0		0		(6)	7.4%
New Generation	31 July 1973	117	160	(2)	1.7%	(1)	0.85%	0		(7)	6.3%
	16 Aug. 1973	135	149	(4)	3.4%	0		0		(35)	26%
	16 Sept. 1973	313	737	(39)	12.4%	(20)	6.4%	(1)	0.3%	(97)	31%
	31 Oct. 1973	284	688	(39)	13.7%	(13)	4.6%	(3)	1.0%	(117)	41%
	12 Dec. 1973	119	122	(21)	17.6%	(6)	5%	0		(45)	38%

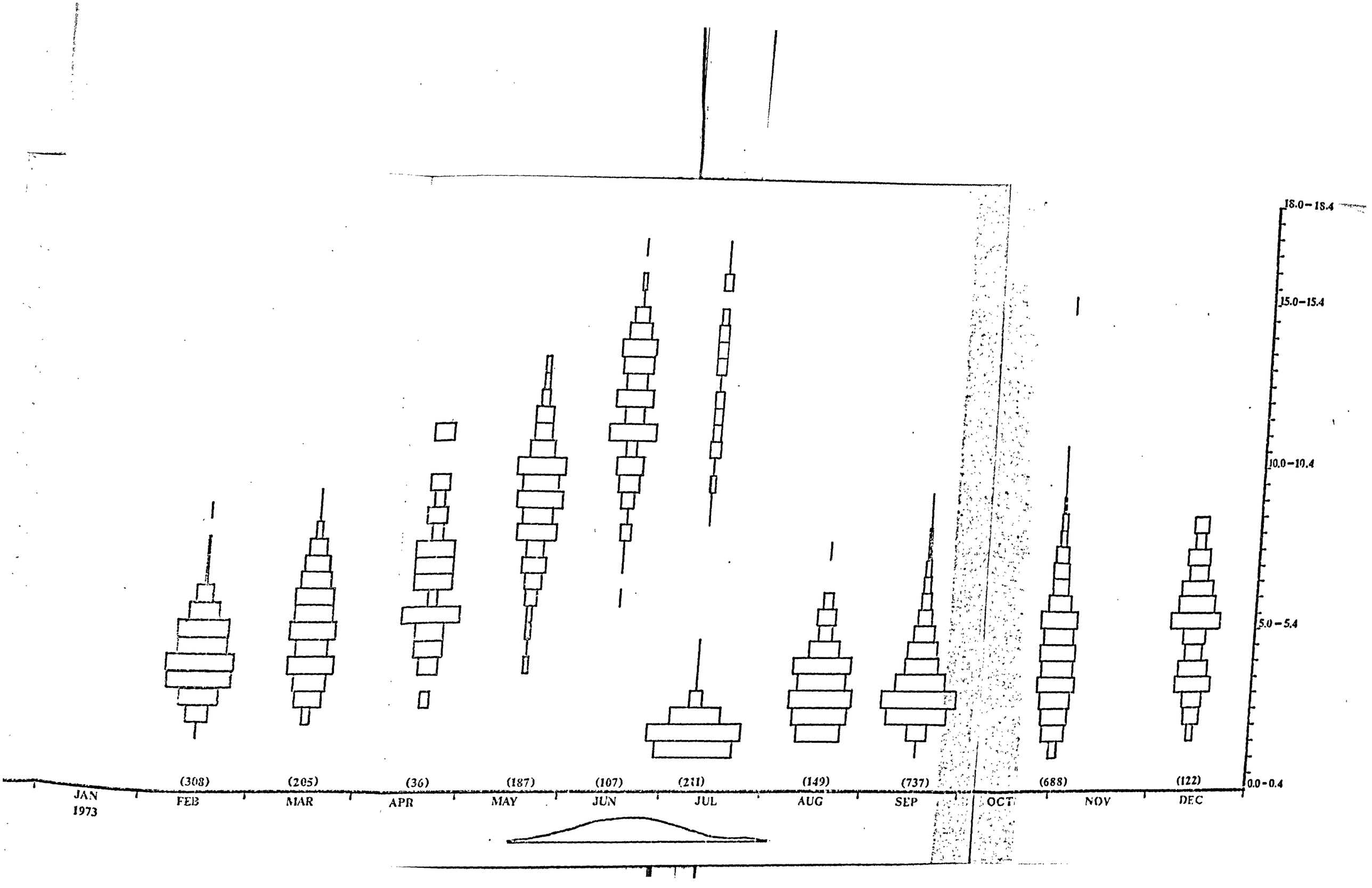
TABLE 27: Numbers of Lymnaea peregra at College Mill Trout Farm containing mature strigeoid infections

Sample Date	Number of snails infected with each strigeoid species				
	Apatemon gracilis	Apatemon minor	Cotylurus cornutus	Diplostomum gasterostei	Diplostomum sp.
9 May 1972	8			6	
30 May	1			1	
14 June	4			2	
8 Sept.	1				
28 Sept.			1		
21 Oct.		3			
9 July 1973					1
16 Sept.		1			
31 Oct.		3			

facing page 196

Figure 58 Shell lengths (in 0.5mm size classes) of Lymnaea peregra from College Mill Trout Farm. Samples were taken from pond 13 during 1972, and from pond 7 during 1973. The number of snails in each sample is shown in parentheses at the foot of each column. The relative abundance of egg masses is indicated along the bottom of the figure.


SHELL LENGTH (0.5 mm SIZE CLASSES)
18.0–18.4
15.0–15.4
10.0–10.4
5.0–5.4
0.0–0.4
= 10 %
(211)
(257)
(253)
(35)
(219)
(196)
(256)
(290)
MAY
JUN
JUL
AUG
SEP
OCT
NOV
EC
1972
EGGS

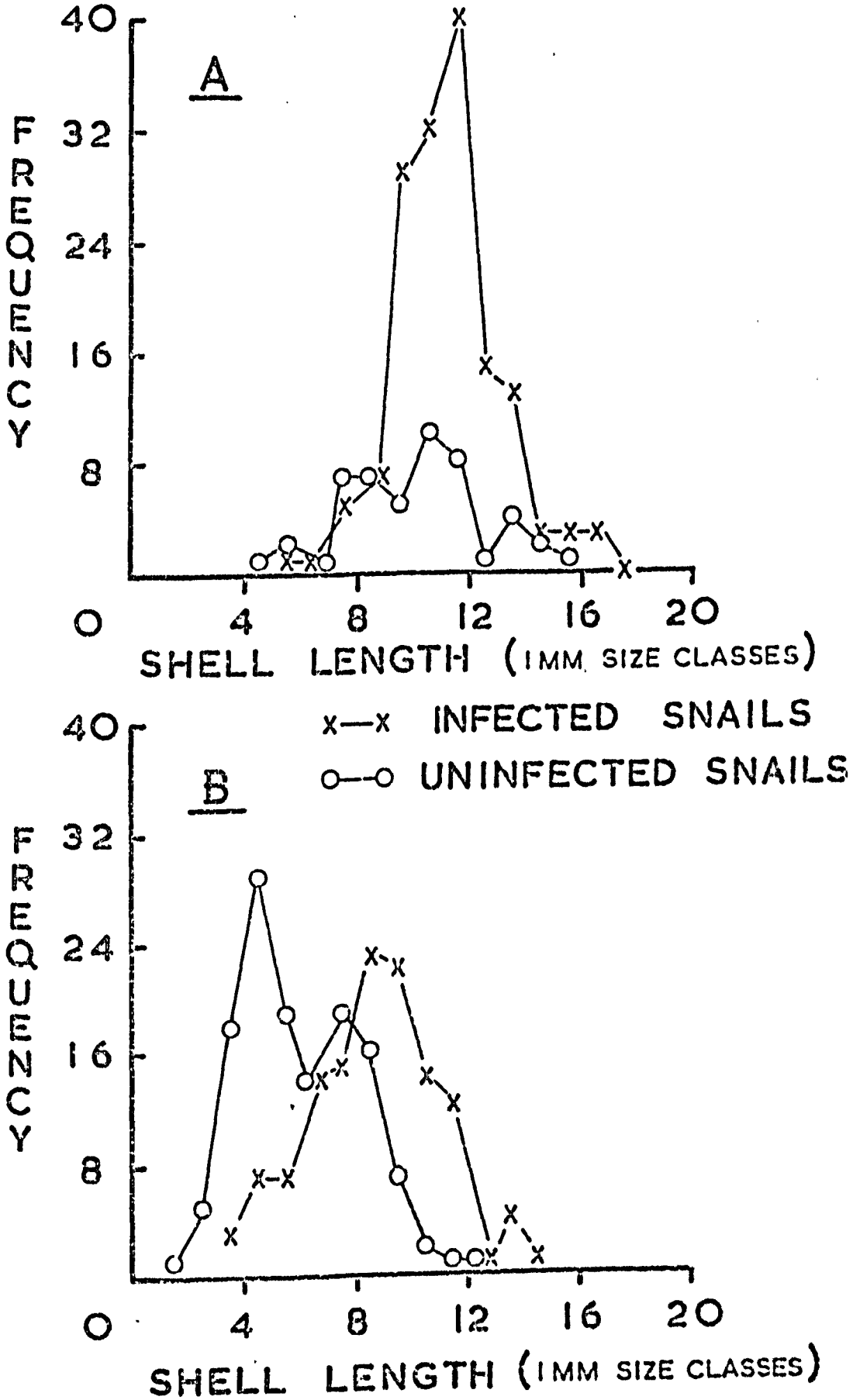

18.0–18.4
15.0–15.4
10.0–10.4
5.0–5.4
0.0–0.4
(308)
(205)
(36)
(187)
(107)
(211)
(149)
(737)
(688)
(122)
JAN
1973
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

facing page 197

Figure 59 Numbers (=frequency) of Lymnaea peregra of different sizes infected with metacercariae of Cotylurus cornutus.

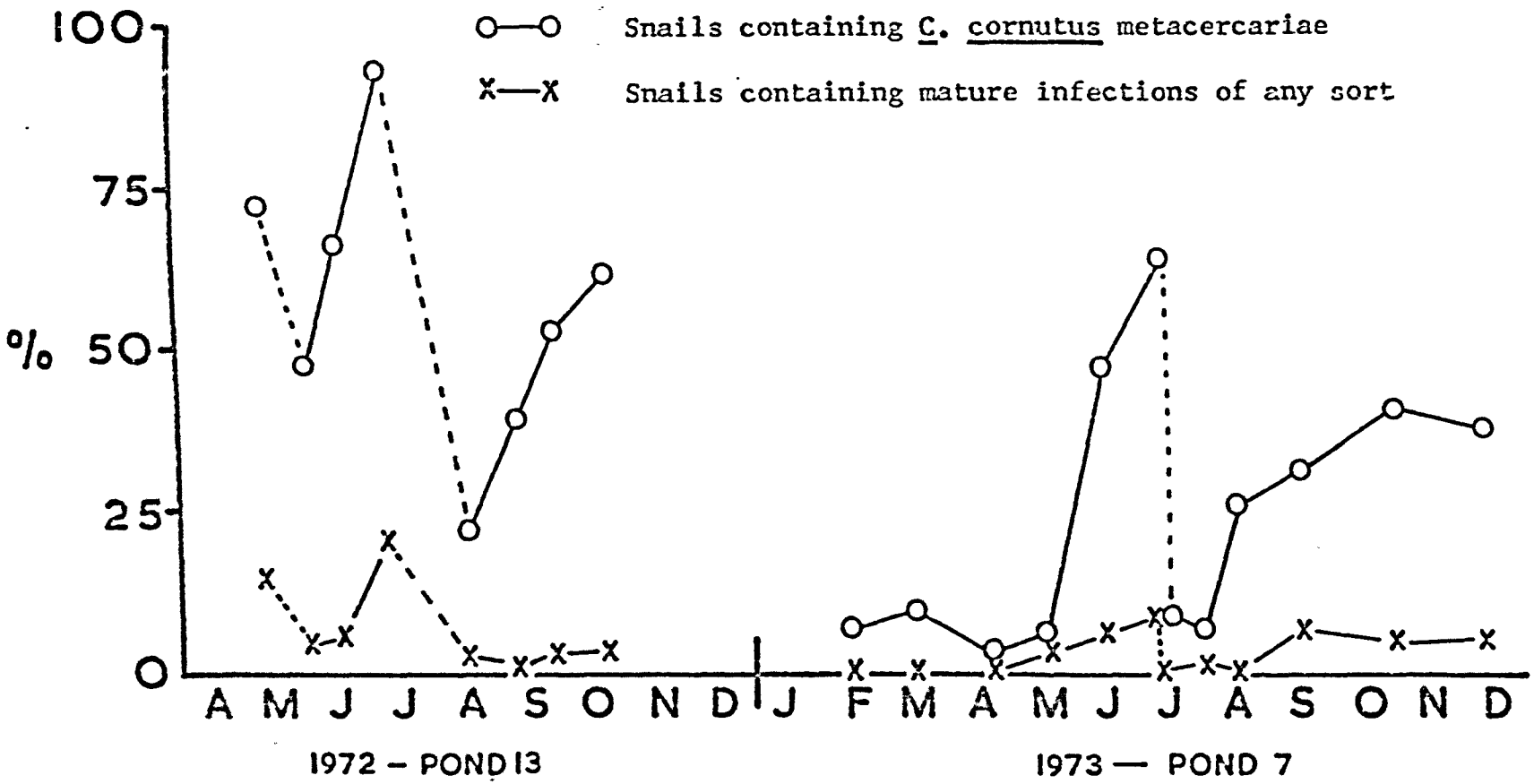
A) Pond 13, 9 May 1972 sample.

B) Pond 13, 30 May 1972 sample.


A
B
FREQUENCY
40
32
24
16
8
0
4
8
12
16
20
SHELL LENGTH (1MM SIZE CLASSES)
x—x INFECTED SNAILS
o—o UNINFECTED SNAILS

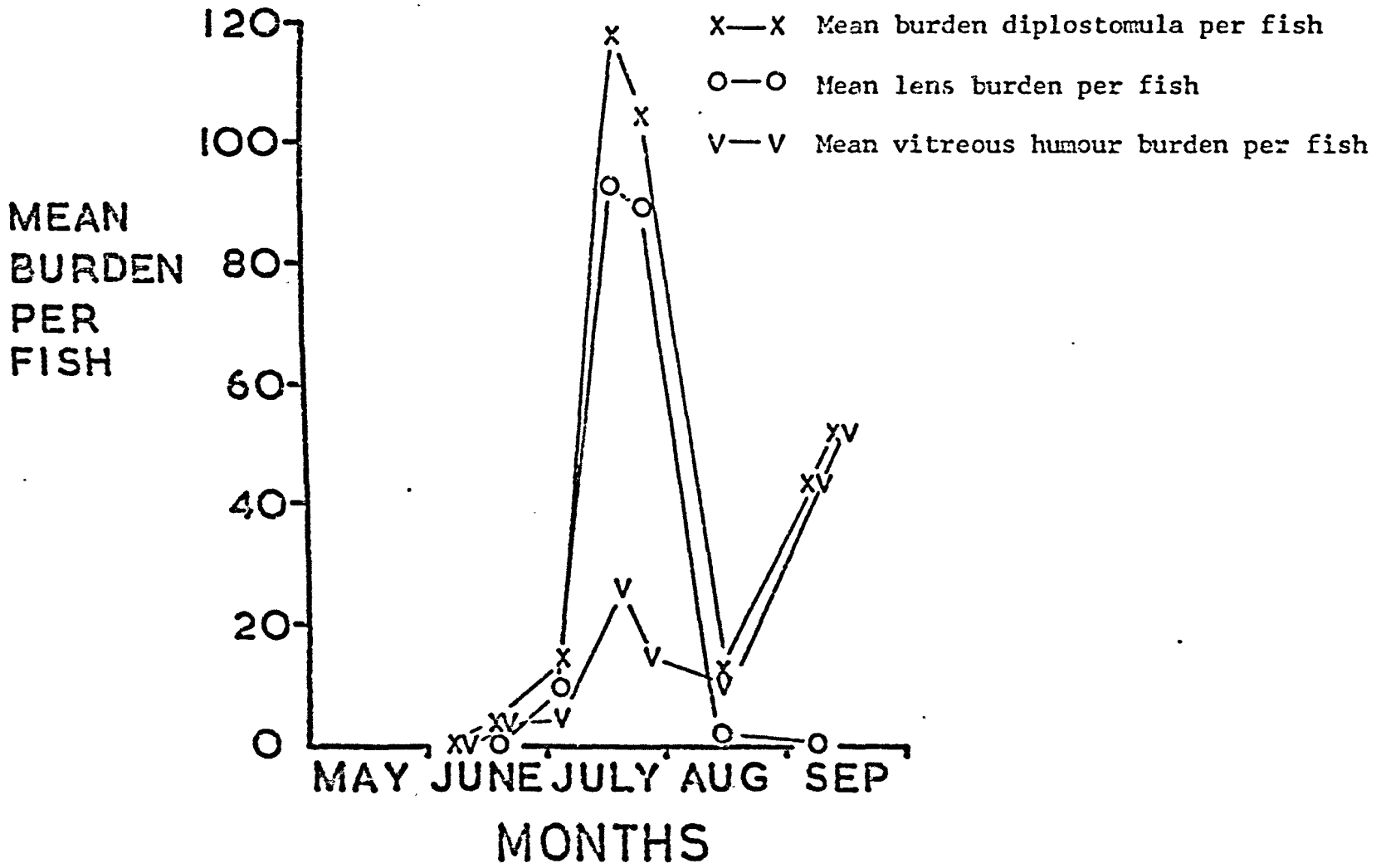
facing page 198

Figure 60 Percentages of Lymnaea peregra from College Mill Trout Farm infected with Cotylurus cornutus metacercariae, or containing mature infections of any sort. Broken lines connect samples thought to represent different generations.


100
75
%
50
25
0
Snails containing C. cornutus metacercariae
Snails containing mature infections of any sort
A M J J A S O N D J F M A M J J A S O N D
1972 – POND 13
1973 — POND 7

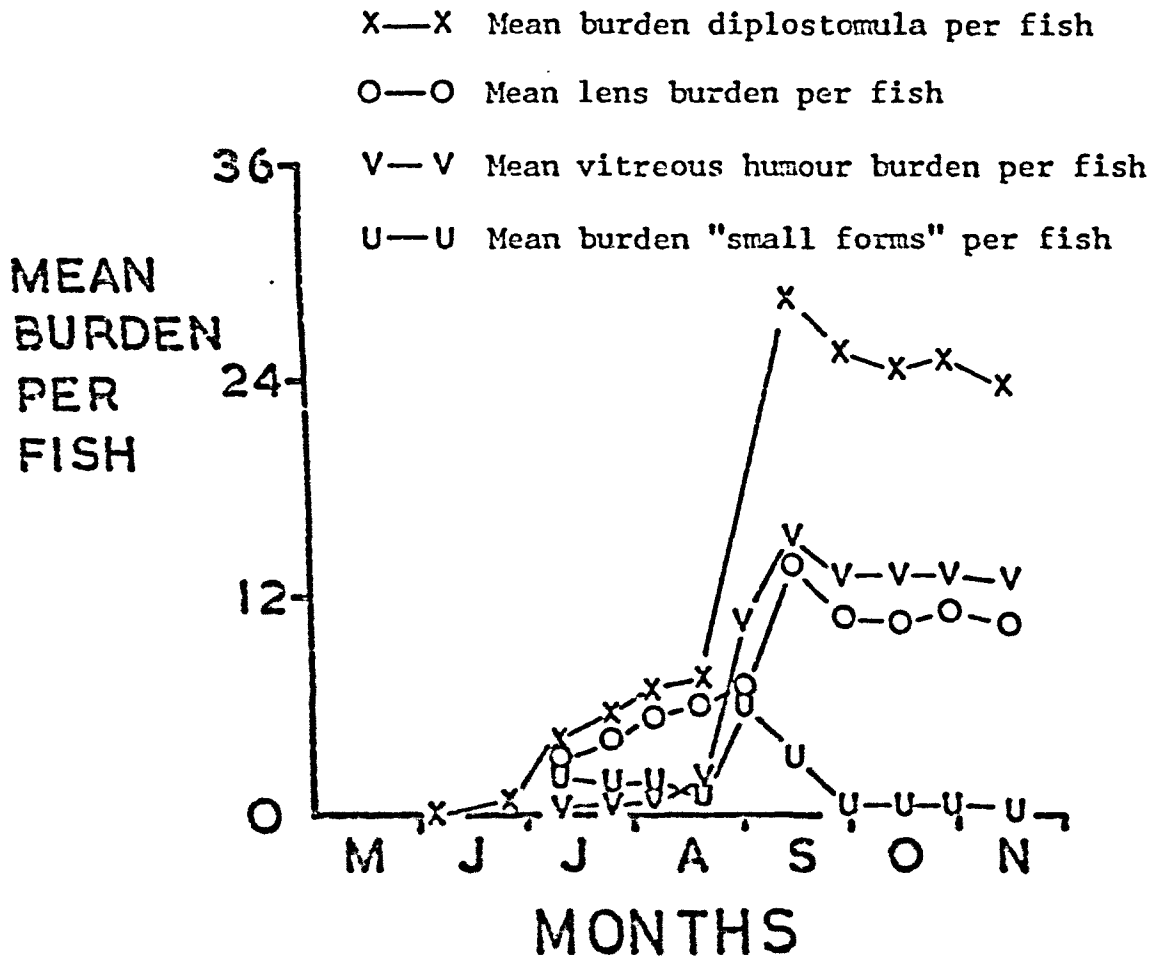
facing page 199

Figure 61 Graph of mean numbers of strigeoid metacercariae, in eyes from samples of 10 rainbow trout, removed at intervals from pond 12 during 1972.


X—X Mean burden diplostomula per fish
O—O Mean lens burden per fish
V—V Mean vitreous humour burden per fish
MEAN BURDEN PER FISH
120
100
80
60
40
20
0
MAY
JUNE
JULY
AUG
SEP
MONTHS

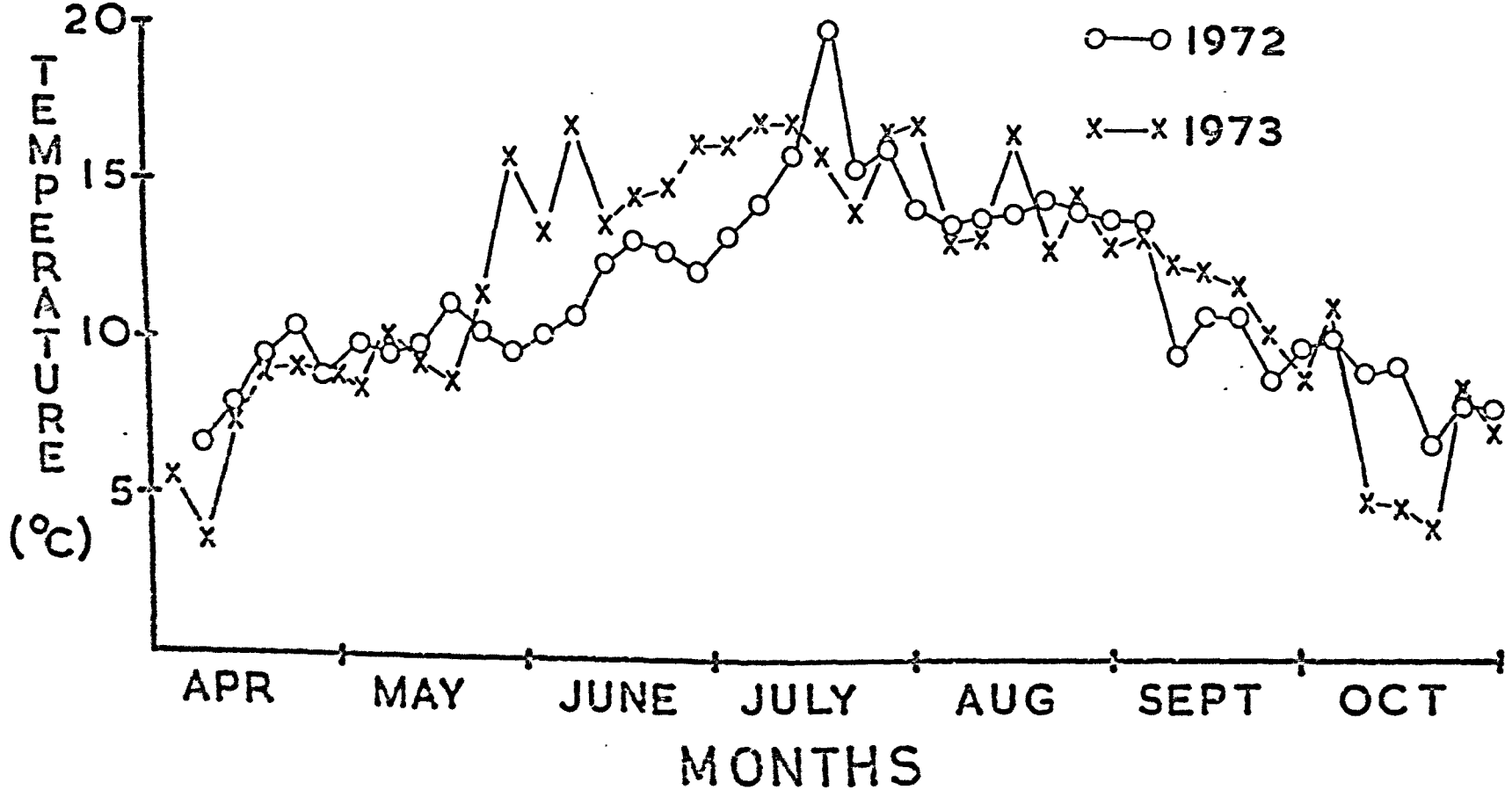
facing page 200

Figure 62 Graph of mean numbers of strigeoid metacercariae , in eyes from samples of 10 rainbow trout, removed at intervals from pond 5 during 1971.


X—X Mean burden diplostomula per fish
O—O Mean lens burden per fish
V—V Mean vitreous humour burden per fish
U—U Mean burden "small forms" per fish
MEAN BURDEN PER FISH
36
24
12
0
M
J
J
A
S
O
N
MONTHS

facing page 201

Figure 63 Water temperatures at the intake lade of College Mill Trout Farm during 1972-1973, recorded in the early afternoon each day. Each point represents an average of the values for 5 days.


20
15
10
5
TEMPERATURE
(°C)
1972
1973
APR
MAY
JUNE
JULY
AUG
SEPT
OCT
MONTHS

CHAPTER 5

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Observations and Experiments on some Larval Trematodes of Freshwater Snails and Fish from Southern Iceland

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During the Glasgow University Iceland Expedition in 1972, freshwater snails and fish in southern Iceland were sampled for larval trematodes. This survey was prompted by the lack of literature concerning larval trematodes there. Indeed, the apparent absence of liver fluke *Fasciola hepatica* (L.1758) from Iceland, (Palsson, pers. comm.) despite the presence of its molluscan and definitive hosts and the low summer temperatures, might lead to the supposition that climatic conditions are too harsh to permit completion of many trematode life-cycles. A number of adult trematodes have been reported from migratory birds in Iceland (Brinkmann 1956), although these parasites could have been acquired elsewhere. However, *Crepidostomum farionis* (Müller, 1784) from the gut of trout and char in Iceland (Brinkmann 1956) presumably completes its life cycle in freshwater there, although this has yet to be demonstrated.

MATERIALS AND METHODS

All field work was done between 14th July and 3rd August, 1972.

Freshwater snails were collected by hand, and placed in individual tubes of water. After some hours in daylight, the tubes were examined for emerged cercariae which were classified as far as possible in the field with the aid of a binocular microscope. Live and preserved (in 10% formalin) snails were dissected to reveal other larval trematodes.

Because of its ease of capture, fish sampling was restricted to the 3-spined stickleback, *Gasterosteus aculeatus L.* Specimens were caught with a dip-net. The eyes, gut and body cavity of a number of fish were examined in the field; others were preserved in 10% formalin for laboratory examination.

Some live fish and snails were brought back to Glasgow for further study. Laboratory raised snails derived from a Scottish

population, and fish caught close to Glasgow, were exposed to infection by placing them in a suspension of cercariae. Trematode eggs, obtained from an experimentally infected duckling by sieving homogenised faeces, were incubated at room temperature. Prior to hatching, embryonated eggs were placed in a tank with uninfected laboratory-raised snails.

Results

During sampling, three species of freshwater snails were found, namely, *Succinea groenlandica* (Beck), *Lymnaea truncatula* (Muller), and *L. peregra* (Muller).

None of 39 *S. groenlandica* from a trickle of water on cliffs two miles east of Vik I Myrdal shed cercariae. Dissection of twenty specimens yielded no trematodes. Similarly, none of 97 *L. truncatula* from pools at the edge of Markarfljotsandur 5 miles north of Storidalur shed cercariae. Forty of these snails were dissected, but no larval trematodes were found. *Lymnaea peregra* is probably the most abundant freshwater mollusc on Iceland (Mandahl-Barth, 1938). It was also the only one found to harbour trematode larvae (Tables I and II). Although representatives of several cercarial groups were noted from this species (Table I), only the morphology and further development of furcocercariae was investigated in detail. All three furcocercariae found were longifurcate pharyngeate distome forms referable to the family Strigeidae.

Table I

Numbers of *Lymnaea peregra* shedding various cercariae

Cercariae shed	Locality			
	Ditch near Reynir, West of Vik	Farm pond at Bolstadur, East of Vik	Hot springs at Landmannalaugar	Heidarvatn
Furcocercaria 1	2	16		
Furcocercaria 2	—			1
Furcocercaria 3	4			
Unclassified Furcocercariae	7			
Double Infections (Furcocercariae plus other)	3	1		
Echinostomes		8		
Monostomes	90	18		2
Xiphidiocercariae				2
Not Shedding	85	113	46	~245
Total	191	156	46	~250

TABLE II

Numbers of dissected *L. peregra* containing larval trematodes

Larval Trematodes	Locality			
	Ditch near Reynir	Farm pond at Holstadur	Hot Springs at Landmannalaugar	Heidarvatn
Metacercariae (Echinostomes and/or *Cotylurus cornutus*)	29	3	4	
Rediae	4	1		
Rediae plus *Cotylurus cornutus* Metacercariae	6			
Strigeid Sporocysts plus *Cotylurus cornutus* Metacercariae	1			
Sporocysts of Xiphidiocercariae				1
Uninfected	2		37	6
Total	42	4	41	7

Furcocercaria 1 has the characteristics of the genus *Apatemon* Szidat, 1928, subgenus *Australapatmon* Sudarikov, 1959 (see Odening, 1969). Other cercariae in this subgenus develop into metacercariae in leeches (Iles, 1960). In an attempt to identify the second intermediate host of furcocercaria 1, a small number of leeches (*Helobdella stagnalis*) were exposed to the cercariae. Although a few cercariae attached briefly to the leeches, none was seen to penetrate, nor were any shed cercarial tails seen. No certain identification of furcocercaria 1 can be made on the basis of this study.

A single snail shedding furcocercaria 2 was found (Table I). This cercaria is referable to the genus *Apatemon*, subgenus *Apatemon* Szidat, 1928, according to the criteria of Odening (1969). It is very similar to the cercaria of *Apatemon cobitidis* Linstow 1890 (= *A. gracilis* Rudolphi 1819) described by Vojtek (1964). The latter possesses four pairs of post-acetabular penetration glands, whereas furcocercaria 2 possesses six pairs. No other major difference between the two was observed.

The identity and life-cycle of this form was established as follows. As part of the work to identify metacercariae in Icelandic sticklebacks, the heads of five fish from Heidarvatn were fed to a duckling. On autopsy after 9 days, one worm was recovered from the small intestine. This was identified as *Apatemon gracilis* (Rudolphi 1819) by Mr. M. T. Harris of the British Museum (Natural History). Eggs obtained from the ducklings' faeces yielded miracidia which infected laboratory raised *Lymnaea peregra*. Cercariae identical with furcocer-

caria 2 were shed from these snails after several weeks. Three-spined sticklebacks (*Gasterosteus aculeatus*) and 10-spined sticklebacks (*Pungitius pungitius* [*L.*]), collected close to Glasgow, were exposed to the cercariae. Several weeks after exposure, the eyes of all the *G. aculeatus* were protruding, and contained large motile metacercariae within the humours. However, none of the *P. pungitius* nor any of a control group of *G. aculeatus* contained these forms. Nine weeks after infection, the eyes of the sole surviving *G. aculeatus* contained cysts grossly visible in the aqueous humour. These were similar to cysts seen in the eyes of *G. aculeatus* from Heidarvatn. No adult worms were obtained when the head of this fish was fed to a duckling.

It is concluded that furcocercaria 2 is the cercaria of *Apatemon gracilis* in Iceland, and that the cyst within the eyes of sticklebacks there represent the metacercaria.

Furcocercaria 3 resembles that described for *Cotylurus cornutus* (Rudolphi 1808) by Szidat (1924) and Dubois (1929). Fifteen laboratory raised *Lymnaea peregra* were exposed to several hundred furcocercaria 3. Nine weeks later, five of the snails were fed to a duckling (*Anas platyrhynchos*). On dissection after 5 days, 60 adult *C. cornutus* were found in the last third of the small intestine.

As well as harbouring cercariae and their precursors, Icelandic *L. peregra* contain numerous metacercariae (Table II). Specimens of *L. peregra* were used in feeding experiments to obtain adult flukes. Three live snails from Reynir were fed to a 5-day-old duckling. At autopsy 13 days later, the small intestine contained 58 *Echinoparyphium recurvatum* (Linstow, 1873), 27 *Hypoderaeum conoideum* (Bloch, 1782), and one *Cotylurus cornutus* (Rudolphi, 1808). In the caeca were found four *Notocotylus attenuatus* (Kossack, 1911). Two more ducklings were fed empty shells of *L. peregra* from the ditch near Reynir. This followed the observation that monostome cercariae, once shed from the snail, promptly form cysts on the shell. In both cases, numerous specimens of *Notocotylus attenuatus* were obtained from the caeca of the ducklings.

The results of field dissections of Icelandic *G. aculeatus* are presented in Table III. All metacercariae found belong to the families Strigeidae and Diplostomatidae. No detailed morphological studies were made, although a number of feeding experiments were carried out.

Two ducklings were each fed five stickleback heads from Heidarvatn. After 5 days, one duckling was autopsied, and three adult

TABLE III

Metacercariae in Sticklebacks

Locality	No. Examined	Metacercariae Found	No. Infected
Pools at edge of Markarfljot	4	None	None
Heidarvatn	11	Cysts (tetracotyle) in humours of eyes.	11
		Diplostomula in retina	11
Frostastadavatn near Landmannalaugar	8	Diplostomula in eye lens	8
		Diplostomula in retina	8

flukes of the genus *Diplostomum, sensu* Dubois, 1970, were recovered from the small intestine. Sections of stickleback eyes from Heidarvatn showed that diplostomula were localised within the retina. Williams (1966) recorded the metacercaria of *Diplostomum gasterostei* Williams, 1966, from this region of the stickleback eye in Scotland. However, without further material, it is not considered possible to assign the Icelandic trematode to this species. Autopsy of the second duckling at day 9 yielded one adult *Apatemon gracilis*. Eggs from this duckling were used in the experimental completion of the life-cycle of furcocercaria 2 (see above).

Two 3-month old black headed gulls (*Larus ridibundus*) failed to yield flukes when fed with stickleback heads from Frostastadavatn.

In the brains of a few preserved fish from both Heidarvatn and Frostastadavatn, diplostomula were found. The identity of these requires further investigation, and fresh material.

DISCUSSION

Of the three snail species examined, only *Lymnaea peregra* contained larval trematodes. The large variety of parasites within *L. peregra* in Iceland reflects the pattern of infection of this species elsewhere. Probert (1966) observed that *L. peregra* was the most heavily parasitised lymnaeid in a Welsh lake. The data in Table I indicates that different populations of *L. peregra* in Iceland are parasitised in differing degrees. The first two localities on Table I, near Reynir, and at Bolstadur, are shallow, static bodies of water containing a rich variety of aquatic life. The snail populations here were the most heavily parasitised. The degree of infection was lower in snails from Heidarvatn, an oligotrophic lake several hundred feet above sea level. The low incidence of larval trematodes in snails from hot springs at Landmannalaugar is of interest. The temperature at which the snails live may be excessive for trematode development.

Tuxen (1944) indicates that *L. peregra* can withstand temperatures over 40°C in hot springs.

The presence of cercariae and metacercariae in snails and fish and the abundance of possible bird definitive hosts is taken as proof that trematode life-cycles can be maintained in Iceland. This appears to be the first report of larval trematodes there.

Of the six genera of trematodes obtained during this work, only two have been recorded previously from Iceland, viz. *Cotylurus cornutus* and *Echinoparyphium recurvatum*. *Apatemon gracilis*, *Diplostomum* sp., *Hypoderaeum conoideum* and *Notocotylus attenuatus* have not been recorded previously.

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GENERAL CONCLUSIONS

Although adult specimens of several species of strigeoids were obtained (Chapter I), most work was done on larval stages, since it was felt (page 37) that these offer a greater array of parameters, morphological and biological, of taxonomic value.

It proved possible, and indeed rather simple, to separate by their morphology cercariae of the four species of Diplostomum studied, when material of all four was available for comparison. It is probably more difficult to identify the cercaria of a single species on its own. This applies most particularly to separating the cercariae of Diplostomum phoxini and D. gasterostei, which have the same resting posture and number of caudal bodies. The bodies (as opposed to the tail-stems) of all the Diplostomum cercariae are very similar, and considerable experience would be needed to distinguish between these.

The most interesting differences between strigeoid species were revealed by exposure of fish to cercariae. Fish species appear to differ in their susceptibility to penetration by cercariae of any given strigeoid. There was evidence that cercariae will penetrate, or attempt to penetrate, any fish they encounter, and that subsequently some species fail to develop (pages 84 and 108). Now that it is clearer what species of strigeoids we have in Scotland, it would be of interest to determine at exactly which stage fish host specificity arises. Such a programme would seek to answer the following questions.

1) Do cercariae of a given species attempt to penetrate all fish they encounter?

This could be answered by the direct observation of cercariae in the presence of fish. *In vitro* studies, using pieces of fish skin stretched across an aperture between two chambers, could also be used. A suspension of cercariae can be placed in contact with the outside of the fish skin, and teleost saline on the inside. Preliminary experiments during this study indicated that such a system would work.

2) Do cercariae injected into the body cavity of fish, thus by-passing the possible barrier of the skin, survive?

Cercariae could also be injected directly into their usual site of localisation (i.e. the eye) to establish whether they can survive in an 'abnormal' host once they have reached their favoured site.

3) Is an immune response by the fish involved?

Cercariae of *Apatemon gracilis* from snails collected at College Mill Trout Farm would only experimentally infect trout, although stone loach and sticklebacks at the trout farm were naturally infected. It was suggested (page 109) that an immune response by the fish may be partly responsible for the failure of most *A. gracilis* cercariae to establish in sticklebacks, and may help dictate location specificity of these that do. Studies of the immune response by fish against parasites are scanty. Further work on this topic may provide some clues as to the nature of host and location specificity of strigeoid

metacercariae.

The immune response in fish is very temperature sensitive. It is possible that low temperatures may favour the establishment of some strigeoids in 'abnormal' hosts, since the fish would be effectively immunosuppressed. It would also be of interest to know whether some strigeoid metacercariae can camouflage themselves with host antigen. If this proves to be the case, transplantation of metacercariae from one fish species to another to test for strain differences (as done by Hoffman, 1958) would give misleading results, since metacercariae would antigenically resemble the original host, and may be destroyed, even if transplanted into another 'normal' host species.

4) Are large fish resistant to infection because of their size (page 109)?

This could be tested by injection of cercariae into large fish at different distances from their favoured site. One of the practical problems preventing such work during this study was the lack of a regular supply of uninfected fish of uniform size.

There is evidence that cercariae of a given species, but from different sources, may differ in their abilities to establish in the same fish host. Although the data obtained is scanty, it suggests that different strains or races may occur in some strigeoid species. Races of D. gasterosteï were obtained as cercariae from the Forth and Clyde Canal, and as metacercariae from perch in Loch

Lomond. Cercariae of each of these races did not infect the normal fish host of the other (Tables 17, 18 and 19). It would be useful to establish both these races in laboratory hosts for further comparative studies. These could include morphological studies, work on the range of fish species penetrated by cercariae of each race, and the development of the metacercariae to the adult, both *in vitro* and *in vivo*. The many laboratory aids to taxonomy could also be employed to compare races, including studies of isoenzymes, nucleic acid hybridisation, genetic studies, and antigenic comparisons.

A major problem encountered during this study concerned the continuous large scale production of cercariae for experimental work. There were times, especially during the winter of 1972/1973, when experimentally infected snails contained many mature cercariae, but none of these would emerge from the snails. The cercariae were normal in morphology, and behaved normally in water if the snail was crushed to release them. It was suspected that some aspect of the snail's physiology was responsible, possibly associated with the short winter day-length. The effects of trematodes on their snail hosts have been reported frequently in the literature. The influence of the diurnal and seasonal rhythms of the snail on its parasites is an almost virgin field. Studies on photoperiodism and on hormonal effects may yield interesting information.

Apart from the season of the year affecting cercarial production, it was also found that experimentally infected snails tended to produce rather few cercariae.

This made it difficult to produce large numbers of freshly shed cercariae at one time for infection experiments. Inadequacies in the diet of the snail may have been responsible for this.

The work on strigeoid eggs reported here suggested that they can develop and hatch in most conditions under which their snail hosts can survive. Further work on the eggs could enter the realms of physiology. It was suggested (Chapter 3) that Diplostomum spathaceum eggs can develop in the absence of oxygen, although further confirmation of this would be desirable. If they should prove to be anaerobes, this would alter some previously held views on trematode eggs (Smyth, 1966 p 72).

A two year study is scarcely sufficient to allow generalisations to be made on the interactions of snail and trematode life-cycles (Chapter 4). Valuable data may be obtained by continuation of such a study over many years. In a situation where snail-borne diseases are of interest to fish farmers and others, the monitoring of snail populations and their trematodes may allow prediction of disease outbreaks. It is regretted that, during this study, regular samples were taken only from ponds in a commercial fish farm, where human influences had to be added to the factors under consideration.

From the point of view of the fish farmer, this study has established that Diplostomum spathaceum is not the only strigeoid of economic importance in Scottish waters. The most important pathogens are probably those infecting the lenses of fish eyes (i.e. D. spathaceum and Diplostomum sp.). Attempts to control these should be directed towards

elimination of snails, as all the strigeoids studied have a single host, Lymnaea peregra, in common. At College Mill Trout Farm, snails within the rearing ponds of the farm itself were responsible for most cercariae in the ponds. Treatment of ponds to kill snails is straightforward, and is already being carried out. If done at the appropriate time (i.e. in June, just before cercariae are first shed), treatment of ponds may prevent serious disease in the fish for the rest of the season.

Elimination of bird hosts is not to be recommended either on practical or legal grounds. Even if the natural definitive hosts were known for all the strigeoids (they are not known for Diplostomum gasterostei and Diplostomum sp.), destruction of most bird species would be illegal. It might prove possible to screen ponds against contamination with bird faeces, but this would not prevent miracidia entering with the water supply.

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Appendix 1 The strigeoids in the classifications of La Rue, Sudarikov, and Dubois.

Classification of La Rue (1957)

Order STRIGEATOIDEA La Rue, 1926

Suborder STRIGEATA La Rue, 1926

Superfamily STRIGEOIDEA Railliet, 1919

Families
- Strigeidae Railliet, 1919
- Diplostomatidae Poirier, 1886
- Cyathocotylidae Poche, 1926
- Proterodiplostomatidae Dubois, 1936
- Bolbocephalodidae Strand, 1935
- Brauninidae Bosma, 1931

Superfamilies
- CLINOSTOMATOIDEA Dollfus, 1931
- SCHISTOSOMATOIDEA Stiles and Hassall, 1926

Suborders
- AZYGIATA La Rue, 1957
- CYCLOCOELATA La Rue, 1957
- BRACHYLAIMATA La Rue, 1957

Classification of Dubois (1953, 1964, 1970b)

Order STRIGEIDA (La Rue, 1926) Odening, 1960 and 1961
name and character amended

Suborder STRIGEATA La Rue, 1926 (= STRIGEIDA Poche, 1926)

Superfamily STRIGEOIDEA Railliet, 1919

Subsuperfamily Strigeoinea Dubois, 1964

Family *Strigeidae* Railliet, 1919

Subfamilies Strigeinae Railliet, 1919
Duboisiellinae Baer, 1938

Subsuperfamily Diplostomatoinea Dubois, 1964

Family Diplostomatidae Poirier, 1886

Subfamilies Diplostomatinae Monticelli, 1888
Alariinae Hall and Wigdor, 1918

Family Proterodiplostomatidae Dubois, 1936

Supersubfamily Proterodiplostomatidi Dubois, 1936

Subfamilies Proterodiplostomatinae Dubois, 1936
Polycotylinae Monticelli, 1888

Supersubfamily Ophiodiplostomatidi Dubois, 1936

Subfamilies Ophiodiplostomatinae Dubois, 1936
Proalarioidinae Sudarikov, 1960

Subsuperfamily Bolbocephalodoinea Dubois, 1970

Family Bolbocephalodidae Strand, 1935

Subfamily Bolbocephalodinae Dubois, 1936

Superfamily CYATHOCOTYLOIDEA (Dubois, 1936) Nicoll, 1937

Subsuperfamily Cyathocotyloinea Dubois, 1970

Family Cyathocotylidae Poche, 1926

Subsuperfamily Prohemistomatoinea Dubois, 1970

Families Prohemistomatidae (Dubois, 1938)
Sudarikov, 1961
Brauninidae Bosma, 1931

Suborders CLINOSTOMATA Allison, 1943
SCHISTOSOMATA La Rue, 1926

Classification of Sudarikov (1959, 1960a and b, 1961)

Order STRIGEIDIDA (La Rue, 1926) Sudarikov, 1959
(= STRIGEIDA Poche, 1926)

Suborder STRIGEATA La Rue, 1926 (= STRIGEOIDEA Railliet, 1919)

Superfamily STRIGEOIDEA Railliet, 1919 (= STRIGEOINEA Dubois)

Families Strigeidae Railliet, 1919
Duboisiellidae Sudarikov, 1959

Superfamily DIPLOSTOMATOIDEA Nicoll, 1937

Families Diplostomatidae Poirier, 1886
Alariidae Tubangui, 1922
Bolbocephalodidae Strand, 1935

Superfamily PROTERODIPLOSTOMATOIDEA Sudarikov, 1959

Families Proterodiplostomatidae (Dubois, 1936)
Hughes, Higginbotham and Clary, 1942
Ophiodiplostomatidae Sudarikov, 1960

Suborder CYATHOCOTYLATA Sudarikov, 1959

Superfamily CYATHOCOTYLOIDEA (Dubois, 1936) Nicoll, 1937

Families Cyathocotylidae Poche, 1926
Brauninidae Bosma, 1931
Prohemistomatidae Sudarikov, 1961

NOTES ON APPENDIX 1

A) Dubois (1964, 1970b) has adopted the order name Strigeida (La Rue, 1926) Odening, 1960 and 1961 name and character amended (= order Strigeatoidea La Rue, 1926, 1957 ex parte). The suffix (-ida) conforms to the endings of the other order names proposed by La Rue (1957), and to the proposals of Article 29 (Recommendation 29A) of the International Code of Zoological Nomenclature, adopted by the 15th International Congress of Zoology (published in 1961). The order is also redefined by Dubois (1970b) to include the three suborders Strigeata La Rue, 1926, Clinostomata Allison, 1943, and Schistosomata La Rue, 1926.

B) Sudarikov (1959) raised the supersuperfamily Strigeida Poche, 1926 to the order rank, proposing the name Strigeidida for this order, and as a new name for the order Strigeatoidae La Rue, 1926. He recognises two suborders, Strigeata La Rue, 1926 and Cyathocotylata Sudarikov, 1959. Dubois (1964) considers the supersuperfamily Strigeida Poche, 1926 a synonym of the suborder Strigeata La Rue. Thus the order Strigeidida of Sudarikov corresponds with the suborder Strigeata La Rue of Dubois. Likewise, the suborder Strigeata La Rue of Sudarikov corresponds with the superfamily Strigeoidea Railliet, 1919 of Dubois. These rank differences for analogous taxa between the classifications of Dubois and Sudarikov extend to the family level and below. The table in this appendix includes the subfamilies only in Dubois' classification.

C) Dubois and Sudarikov disagree as to the status of the

families Bolbocephalodidae Strand, 1935 and Proterodiplostomatidae Dubois, 1936. Dubois (1970b) placed the former family in a distinct subsuperfamily, the Bolbocephalodoinea (Dubois, 1936) Dubois, 1970 (a taxon analogous with a superfamily in the classification of Sudarikov, see Note B on previous page). Sudarikov (1960b) considered that the Bolbocephalodidae should be placed in the superfamily Diplostomatoidea, and that Dubois (1936) had been incorrect in creating a separate suprafamilial taxon for the family. Sudarikov also proposed to remove the Proterodiplostomatidae to a separate superfamily (designated Proterodiplostomatoidea Sudarikov, 1959). This was intended to emphasise the differences in final hosts between the Proterodiplostomatidae (parasites of reptiles) and the Diplostomatidae (parasites of birds and mammals).

APPENDIX 2

Definitions of the families Strigeidae Railliet, 1919 and Diplostomatidae Poirier, 1886, with lists of their genera. Based on Dubois (1968a, 1970a snd b).

An asterisk denotes genera or subgenera for which life-cycles are wholly or partly known.

Family Strigeidae Railliet, 1919.

Strigeoids; parasitic as adults in birds and mammals; paraprostate lacking; forebody cup shaped, bearing two lateral lappets and a holdfast, the two lips of which are situated one dorsally, the other ventrally, and separated by a deep slit; proteolytic gland generally distinct, situated at the junction of the forebody and hindbody; ovary generally kidney-shaped in dorsal view; testes situated one behind the other, and of various shapes; known metacercariae belong to the larval genus Tetracotyle de Filippi, 1855 parasitising snails, leeches, and all classes of vertebrates.

Subfamily Strigeinae Railliet, 1919

Strigeidae parasitic in birds as adults; vitellaria in both fore- and hindbody, or confined to hindbody.

Tribe Strigeini Dubois, 1936 ex Railliet, 1919

Strigeinae with vitellaria in both fore- and hindbody. Genera; Apharyngostrigea*Ciurea, 1927; Chabaustrigea Sudarikov, 1959; Ophiosoma Szidat, 1928; Strigea* Abildgaard, 1790; Parastrigea* Szidat, 1928.

Tribe Cotylurini Dubois, 1936

Strigeini with vitellaria confined to the hindbody. Genera; Apatemon Szidat, 1928 (subgenera Apatemon* Szidat,

1928; Australapatemon* Sudarikov, 1959); Cardiocephalus* Szidat, 1928; Cotylurus Szidat, 1928 (subgenera Cotylurus* Szidat, 1928; Ichthyocotylurus* Odening, 1969); Nematostrigea Sandground, 1934; Pseudapatemon Dubois, 1936; Schwartzitrema Vigueras, 1941.

Subfamily Duboisiellinae Baer, 1938

Strigeidae parasitic as adults in mammals; vitellaria confined to forebody.
Genus; Duboisiella Baer, 1938.

Family Diplostomatidae Poirier, 1886

Strigeoids parasitic as adults in birds or mammals; lacking a paraprostate; forebody flattened, leaf-shaped or spatulate, bearing a holdfast of definite shape, with or without a central cavity, and with the proteolytic gland underlying it; ovary of various shapes; testes of various shapes, symmetrical or asymmetrical; known metacercariae belong to larval genera Diplostomulum Brandes, 1892 and Neascus Hughes, 1927 parasitic in all classes of vertebrates.

Subfamily Diplostomatinae Monticelli, 1888 ex Poirier, 1886

Diplostomatidae parasitic as adults in birds; vitellaria in both fore- and hindbody, or confined to hindbody; holdfast generally small to medium sized, with a median slit, and not greater in diameter than half the length of the forebody; life-cycle without a mesocercarial stage.

Tribe Diplostomatini Dubois, 1936 ex Poirier, 1886

Diplostomatinae with vitellaria in fore- and hindbody.
Genera; Bolbophorus* Dubois, 1935; Diplostomum von Nordmann, 1832 (subgenera Diplostomum* von Nordmann, 1832; Tylodelphys* Diesing, 1850; Adenodiplostomum Dubois, 1937;

Glossodiplostomoides Bhalerao, 1942; Austrodiplostomum* Szidat and Nani, 1951; Dolichorchis* Dubois, 1961); Harvardia Baer, 1932; Hysteromorpha* Lutz, 1931; Lophosicyadiplostomum Dubois, 1936; Mesoophorodiplostomum* Dubois, 1936; Neodiplostomum Raillict, 1919 (subgenera Neodiplostomum* Railliet, 1919; Conodiplostomum* Dubois, 1937); Neoharvardia R. Gupta, 1963; Ornithodiplostomum* Dubois, 1936; Posthodiplostomoides* M.O. Williams, 1969 Posthodiplostomum* Dubois, 1936; Sphincterodiplostomum* Dubois, 1936; Procrassiaphiala Verma, 1936.

Tribe Crassiphialini Dubois, 1936

Diplostomatinae with vitellaria largely or entirely confined to hindbody.
Genera; Allodiplostomum Yamaguti, 1935; Cercocotyla Yamaguti, 1939; Crassiaphiala* Van Haitsma, 1925; Pseudodiplostomum Yamaguti, 1934; Pulvinifer Yamaguti, 1933; Scolopacitrema Sudarikov and Rykovsky, 1958; Subuvulifer Dubois, 1952; Uvulifer* Yamaguti, 1934

Tribe Codonocephalini Sudarikov, 1959

Diplostomatinae with vitellaria confined to the hindbody; aberrant holdfast; progenetic metacercaria in frogs.
Genus; Codonocephalus* Diesing, 1850

Subfamily Alariinae Hall and Wigdor, 1918

Diplostomatidae parasitic as adults in mammals; Vitellaria largely or entirely confined to the forebody, tending to accumulate in the holdfast which is often greatly enlarged; life-cycle includes a mesocercarial stage.
Genera; Alaria Schrank, 1788 (subgenera Alaria* Schrank, 1788; Paralaria* Krause, 1914); Cynodiplostomum Dubois, 1936; Didelphodiplostomum* Dubois, 1944; Fibricola*

Dubois, 1932; Pharyngostomoides* Harkema, 1942; Pharyngostomum* Ciurea, 1922; Podospathalium Dubois, 1932; Procyotrema Harkema and Miller, 1958; Prudhoella Beverly-Burton, 1960.

APPENDIX 3

Definitions of strigeoid larval genera - from Hoffman (1960).

Larval genus Diplostomulum Brandes, 1892

1) Forebody foliaceous, concave ventrally.
2) Hindbody a small conical prominence on the postero-dorsal part of the forebody.
3) Reserve excretory system of more or less definitely arranged tubules with calcareous corpuscles, round or ellipsoidal, disposed in vesicles at termini of small branches.
4) Usually a pair of lateral pseudosuckers on the antero-lateral edges of the forebody, beside the oral sucker.
5) No true cyst of parasite origin (except in Bolbophorus confusus (Krause, 1914) and in Dolichorchis spp.).
6) Known adult species belong to the family Diplostomatidae Poirier, 1886.

Larval genus Tetracotyle de Filippi, 1855

1) Forebody oval or ovate-oblong in contour, and relatively thick, concave ventrally, or cup-shaped.
2) Hindbody a short rounded prominence at posterior end of forebody, sometimes incospicuous.
3) Reserve excretory system a large continuous space occupying dorsal and lateral regions of the forebody, with a sheet-like extension into the ventral lip of the anterior suctorial pocket; small bodies in reserve excretory vessels, mainly in the anterior part of the worm.
4) A pair of lateral pseudosuckers on anterolateral edge beside oral sucker.

5) A true cyst of parasite origin.
6) Known adult species belong to the family Strigeidae Railliet, 1919.

Larval genus Neascus Hughes, 1927

1) Forebody much like Diplostomulum.
2) Hindbody more extensively developed than in Diplostomulum.
3) Reserve excretory system more extensively developed than in Diplostomulum; with calcareous corpuscles not confined to termini of small branches, which do not end blindly, but constitute anastomoses.
4) No lateral pseudosuckers.
5) Generally encysted with true cyst of parasite origin.
6) Known adult species belong to the family Diplostomatidae Poirier, 1886.

APPENDIX 4

Diagnoses, where available, of those genera and species listed in Chapter 1. Based on Dubois, 1968a, 1970a Niewiadomska, 1971a and Odening, 1969.

Genus Apatemon Szidat, 1928

Cotylurini with bisegmented body; pharynx present; forebody variously cup-shaped, lacking lateral extensions, small multilobed proteolytic gland, lacking vitellaria. Hindbody sac-shaped, subreniform or subcylindrical; generally curved and lacking a collar, separated from the forebody by a constriction. Ellipsoidal or reniform ovary in the anterior 2/5 ths. of the hindbody; testes of various shapes and generally oriented obliquely, may be bi- or trilobed (with the lobes directed anteriorly) or multilobed. Copulatory bursa of medium size, with a terminal opening containing a genital cone at the base of which the uterus and ejaculatory duct unite to form the hermaphroditic duct.

Subgenus Apatemon Szidat, 1928

Apatemon with poorly developed genital cone traversed by a narrow, straight, hermaphroditic duct with little associated musculature. Cercariae with rudimentary gut caeca; six, eight, or more postacetabular penetration gland cells. Excretory formula 2[(2)+((2)+(1))]= 10; postacetabular excretory commissure; metacercariae in fishes.

Apatemon (A.) gracilis (Rudolphi, 1819) Szidat, 1928

Length to 2.3 mm. Forebody (350-700/250-540 μm); hindbody (600-1800/200-600μm) separated from the forebody

by a distinct constriction; tapering towards the rear. Ratio hindbody/forebody length = 1.9-3.0. Oral sucker terminal (85-180/70-140 μm), followed by a small pharynx (45-80/40-80 μm); ventral sucker postequatorial (100-255/90-245 μm). Ovary oval (70-130 μm long), situated in hindbody between 22 and 40% of the length of the hindbody. Testes possess two large lobes directed antero-dorsally, and occupy the second or third quarters or the third and fourth fifths of the hindbody, first testis oriented obliquely (170-380 μm long), second longer (190-435 μm long). Vitellaria most dense ventro-laterally in hindbody, especially anterior to the ovary, but reaching almost to the posterior of the body. Copulatory bursa rather small, with a terminal pore lacking a distinct muscular sphincter; genital cone small (210/150 μm), traversed by a narrow hermaphroditic duct.

Subgenus Australapatemon Sudarikov, 1959

Apatemon with well developed and muscular genital cone, traversed by a muscular, and often convoluted, hermaphroditic duct. Cercaria with gut terminating in two long caeca. Eight (rarely six) postacetabular penetration gland cells. Excretory formula 2[(2)+((4)+(1))] = 14; with one or two excretory commissures. Metacercariae in leeches.

Apatemon (Australapatemon) minor Yamaguti, 1933

Length to 2.5 mm. Forebody (250-870/280-630 μm); hindbody (540-1730/250-670 μm), separated from the forebody by a distinct constriction. Ratio hindbody/forebody length = 1.4-2.6. Oral sucker terminal or subterminal (80-145/60-130 μm), followed by a much

smaller pharynx (40-65/33-65 μm). Ventral sucker equatorial or postequatorial (92-198/80-198 μm). Ovary oval, (66-135 μm long), situated in hindbody between 13 and 28% along the length of the hindbody. Testes lobed, occupying the second and third quarters of the hindbody; first testis oriented obliquely (99-306 μm long), the second a little longer (130-408 μm long). Vitellaria extend ventrolaterally in the hindbody from the junction of the two segments, but do not completely mask the genital cone. Copulatory bursa of medium size, with a distinct but small genital cone (150-280 μm long). Hermaphroditic duct broad, and transversely folded when the genital cone is retracted.

Genus Cotylurus Szidat, 1928

Cotylurini with bisegmented body; pharynx present. Forebody variously cup-shaped or hemispherical, lacking lateral extensions; Proteolytic gland poorly developed and rather diffuse; forebody may contain a few vitellaria. Hindbody variously cylindrical, lacking a collar, and separated from the forebody, to which it is often attached excentrically, by a distinct constriction. Ellipsoidal or reniform ovary situated in the anterior half of the hindbody. Testes may be trilobed with the lobes directed posteriorly (one dorsal and two lateral), or multilobed. Copulatory bursa of moderate size with a subterminal dorsal pore; genital cone absent, but a genital bulb present, at the base of which the genital ducts emerge dorsally into the copulatory bursa.

Subgenus Cotylurus Szidat, 1928

Testes with three lobes. Cercariae with two pairs

of preacetabular penetration gland cells. Flame cell formula 2[(2+2)+((2+2)+(2))] = 20, preacetabular excretory commissure present. Metacercariae in snails and leeches.

Cotylurus cornutus (Rudolphi, 1808) Szidat, 1928

Length to 2.75 mm. Forebody hemispherical to cup-shaped, broader than long (300-720/340-800 µm), or may be globular in a state of contraction. Hindbody (900-2100/380-740 µm), attached excentrically to the forebody, from which it is separated by a distinct constriction; maximum diameter at level of testes, slightly tapered posterior to these. Ratio hindbody/forebody length = 2.4-4.4. Oral sucker marginal (65-155/65-140 µm), much smaller than the equatorial or postequatorial ventral sucker (100-200 µm). Pharynx (45-110 µm). Ovary spherical or oval, situated in the hindbody between 16 and 34% along the length of the hindbody. Testes rather large and trilobed, lobes directed posteriorly; first testis (225-500 µm long), second testis (270-570 µm long). Vitellaria entirely confined to the ventro-lateral region of the hindbody, extending posteriorly to the beginning of the copulatory bursa. Copulatory bursa of medium size, containing a genital bulb.

Subgenus Ichthyocotylurus Odening, 1969

Testes trilobed or multilobed. Cercariae with two pairs of postacetabular penetration gland cells. Excretory formula 2[(2+2)+((2+2)+(2))] = 20, preacetabular excretory commissure. Metacercariae in fishes.

Cotylurus (Ichthyocotylurus) variegetus (Creplin, 1825) Szidat, 1928, sensu Odening and Bockhardt, 1971.

No diagnosis available from the literature.

Genus Diplostomum von Nordmann, 1832

Diplostomatini, with the body bisegmented to a greater or lesser extent; anterior extremity bears lappets; holdfast circular or (more rarely) elliptical in outline, with a median slit. Ovary anterior to testes. Shape and structure of testes varying between subgenera. Metacercariae of the larval genus Diplostomulum Brandes, 1892.

Subgenus Diplostomum von Nordmann, 1832

Diplostomum with distinctly bisegmented body; first testis asymmetrical; copulatory bursa lacking a genital cone. Cercariae posses four pairs of postacetabular penetration gland cells. Calcareous bodies in the reserve excretory system of the metacercaria are spherical in shape. Metacercariae in fish and cyclostomes.

Diplostomum (D.) spathaceum spathaceum (Rudolphi, 1819) Braun, 1893

Length to 4.45 mm. Forebody flattened, oval or elliptical in outline (600-1800/270-960 μm), broadest at the level of the holdfast; anterior extremity trilobed, bearing the oral sucker and lappets. Hindbody (500-3220/190-720 μm) attached to the forebody close to the posterior edge of the dorsal face of the latter, rather narrow at this junction, but broader toward the testes. Ratio hindbody/forebody length = 0.66-2.62. Pharynx elliptical (16-91/10-75 μm), often similar in length to the oral sucker (20-100/20-104 μm). Ventral sucker larger (23-110/20-140 μm), situated between 47 and 60% along the length of the forebody. Holdfast circular or elliptical in outline (55-450/33-390 μm), often slightly protruding; bilobed proteolytic gland lying under posterior extremity

of holdfast. Ellipsoidal ovary (20-255/23-235 μm) situated laterally in hindbody between 25 and 58% along the length of the hindbody. Testes concave ventrally, situated in the fourth and fifth sixths of the hindbody; first testis asymmetrical (40-460/50-650 μm), and the second bilobed (40-485/50-650 μm), occupying the entire width of the body. Vitellaria occur in the forebody to the anterior edge of the holdfast, but are most dense in the hindbody. Copulatory bursa small with a subterminal pore.

Diplostomum (D.) phoxini (Faust, 1918) Arvy and Buttner, 1954

Length to 1.42 mm. Forebody elliptical (320-700/300-600 μm), often deeply concave ventrally. Anterior extremity trilobed, bearing the oral sucker and lappets. Hindbody (290-720/220-400 μm) short and cylindrical or subconical, recurved dorsally, and often slightly shorter than the forebody, from which it is separated by only a slight constriction. Ratio hindbody/forebody length = 0.60 - 1.03. Pharynx ellipsoidal (48-70/31-45 μm), slightly shorter than the oral sucker (50-72/40-80 μm); ventral sucker (50-80/70-96 μm) situated between 50 and 66% along the length of the forebody. Holdfast circular in outline (90-180/90-160 μm). Ellipsoidal ovary, median or submedian, situated at the junction of the segments. Testes occupying the first half or two-thirds of the hindbody; first testis asymmetrical (54-190/125-220 μm), the second bilobed, and concave ventrally (72-300/180-360 μm). Vitellaria abundant in the forebody, and often extending anterior of the ventral sucker; less abundant in the hindbody. Copulatory bursa small with a subterminal pore, and a muscular wall.

Diplostomum (D.) gasterostei Williams, 1966

Dubois (1970a p 310) considers the diagnosis for D. phoxini to suffice for this species also. However, specimens of D. gasterostei obtained in this study greatly exceed (Table 6) the dimensions quoted by Dubois for D. phoxini .

The copulatory bursa of D. gasterostei lacks the muscular wall reported for D. phoxini (see Dubois, 1970a p 310).

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